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Title page, September, third title, *read* probable *for* possible; fourth title, *add* of apple.

Page 9, DIPPENAAR abstract, paragraph 2, line 2, *read* .02 per cent for 02 per cent; *read* $HgCl_2$ *for* $HgCL_2$; *read* HCl *for* HCL.

Page 22, last line, *read* was *for* were.

Page 83, paragraph 2, lines 2 and 3, *read* SPRAGUE and HEALD *for* HEALD and SPRAGUE.

Page 90, citation 5, *read* SPRAGUE, R., and F. D. HEALD *for* HEALD, F. D. and R. SPRAGUE. *After* of (*in title*), *add* the.

Page 234, table, column 1, last paragraph, *read* for 19 *for* of 19.

Page 239, line 6 from bottom, *read* systemically *for* systematically.

Page 271, last line, *read* PARSON *for* PARSONS.

Page 362, line 16, *read* direction of the force, applied at the edge *for* direction of the applied force, *i.e.*, the edge. Lines 17 and 19, *read* plane *for* place.

Page 392, last paragraph, line 1, *read* diseases *for* disease.

Page 431, paragraph 2, line 5, *read* 35.6° in two experiments, this temperature is near the maximum *for* infection. In . . . for 35.6° in 11 days at 8°. . . .

Page 472, Summary, paragraph 2, line 4, *after* sugar *add* beet.

Page 602, paragraph 3, line 5, *read* 2.68 μ *for* 2.88 μ.

Page 608, paragraph 2, line 8, *read* did not suffice *for* sufficed.

Page 620, *transpose* line 10 to end of third line from bottom.

Page 785, Summary, paragraph 3, *read* curtisii *for* curtissii.

Page 800, for line 3 *substitute* period the temperature varied considerably and the humidity remained.

Page 824, line 8, *delete* quotation marks *after* uncommon; line 9, *add* quotation marks *after* 1827–1831.

Page 962, Summary, last paragraph, line 3, *read* July and August *for* June and July.

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Heterothallism in Puccinia graminis, P. coronata, and Melampsora lini. RUTH F. ALLEN.

Puccinia graminis and *P. coronata* are heterothallic. The isolated, unisexual infection makes good vegetative growth and produces active spermogonia and receptive hyphae, but the sporophyte is not initiated and aeciospores do not form. Only when spermatia of one sex are placed upon the surface of an infection of opposite sex or when adjoining infections of opposite sex become confluent does the normal development of aecia take place.

In *P. coronata* hyphae from vegetative mycelium, from spermogonia, and from aecial primordia grow out into stomata or between epidermal cells and serve to receive spermatial nuclei. In *P. graminis* formation of receptive hyphae is sharply limited by the tough epidermis of the barberry leaf. The spermogonia themselves serve as the main port of entry for spermatial nuclei.

Flax rust, *Melampsora lini,* also is heterothallic. The isolated infection bears no aeciospores. Fertilization may occur in infection from 8 to 20 days old, rarely older. Cells of the gametophytic, vegetative mycelium contain 1 to 4 nuclei. The young spermogonium centers on a stoma and at first uses the stomatal aperture as an ostiole. At this time no hyphae reach the surface of the leaf except at spermogonia. Cell fusions in the aecium occur regularly.

(Cooperative investigations, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the California Agricultural Experiment Station.)

Serologic reaction as a means of determining the concentration of tobacco mosaic virus. HELEN PURDY BEALE.

Green-leaf extracts of Turkish tobacco affected with mosaic (Johnson's No. 1 virus) were tested for their ability to form precipitates when mixed with serum of rabbits hyperimmunized against homologus extracts. The concentration of active virus in each extract was then determined independently by the number of local lesions resulting from inoculation of *Nicotiana glutinosa,* according to the method of Holmes (Bot. Gaz. 87: 39–55. 1929). A correlation was found to exist between the virus concentration of the various extracts and their ability to precipitate antiserums. It is suggested that the precipitin reaction may be of value for rapid and accurate determination of the relative concentration of virus extracts.

A fruit spot associated with the papular type of apple measles. ANTHONY BERG.

From several specimens of Grimes Golden apples bearing numerous circular, nearly black fruit spots, a fungus was isolated that in every respect resembles the fungus producing the papular type of "apple measles" on the wood. By artificial inoculations upon fruits of Rome, similar spots were produced, infection taking place through the lenticels. Although no inoculations on apple twigs have so far been made, it is pertinent that the infected fruits were collected by the Department of Botany, Purdue University Agricultural Experiment Station, in an orchard known to be infected with the papular type of apple measles.

Electrophoretic studies on purified plant viruses. J. M. BIRKELAND.

Our experiments indicate that the direction of migration of the viruses in an electric field is not altered by the state of purity nor the method of purification. The crude viruses of tobacco mosaic, spot necrosis, ring spot, and attenuated mosaic were found to migrate to the anode; that is, they behaved as though negatively charged. The same viruses, purified according to the methods described by Vinson and Petre and

by Brewer, Kraybill, and Gardner, also migrated to the anode. The purified preparations were biuret-negative.

Experiments on acquired immunity in tobacco mosaic and spot necrosis. J. M. BIRKELAND.

Results of our experiments indicate that *Nicotiana glutinosa* does not develop an acquired immunity from infection with the virus of tobacco mosaic or of spot necrosis. One series of plants was tested 4 weeks and another series 8 weeks after the initial infection. The test inoculation was made on new leaves and the number of local lesions were used as an indication of susceptibility. There was no significant difference in the number of lesions produced on the infected plants and on the controls. Plants infected with tobacco mosaic and tested with spot necrosis, as well as plants infected with spot necrosis and tested with tobacco mosaic, gave similar results. In some instances there seemed to be a greater amount of secondary necrosis around the primary lesion on the infected plants than on the controls.

Total amount of copper applied and ratio of lime to copper in Bordeaux as important factors in potato spraying. F. M. BLODGETT, E. O. MADER, O. D. BURKE, and R. B. MCCORMACK.

In potato-spraying experiments for 4 years at Pittsford, N. Y., on Rural New Yorker potatoes, there is strong evidence that the total amount of copper applied is an important factor in increasing yields of potatoes. Maximum yields were obtained by the application of from 70 to 90 lbs. of copper sulphate per acre per season in the form of Bordeaux, whether the variation was made by size or nozzle openings to apply more or fewer gallons per acre, by concentration of mixture, or by frequency of applications. At Riverhead and Sagaponack on Long Island, with Green Mountain potatoes the influence of the total amount of copper applied was not so marked and indicated that not over 40 to 50 lbs. per season are required to give maximum results.

In comparing Bordeaux mixtures made with different kinds of lime, that made with high magnesium lime has given slightly, but fairly consistently, better results than the mixture made with high calcium lime. As the magnesium is highly insoluble and inactive, the results seemed to indicate that a too high ratio of lime to copper was used. Field experiments in the 3 localities in the current season seem to confirm this explanation.

Development of apple scab on stored fruit. C. O. BRATLEY.

The demand for the present studies came as a result of frequent controversies regarding the responsibility for apparent increase of scab on commercially stored fruit. Experiments conducted during the past 5 years have shown that old lesions may enlarge and new ones appear on fruit while stored at 32° F., but that factors operative in the orchard are of more importance than those in storage.

Both enlargement of old lesions and development of new ones were favored by higher temperatures and humidities. Neither the use of different types of storage containers nor delay in storing fruit had any effect, except as the humidity and temperature were varied.

In some groups of scab-free fruit as many as 50 per cent of the apples developed new lesions during storage. Fruit picked after a wet fall from poorly sprayed, heavily infected trees was particularly susceptible.

All attempts to obtain spread from scabby to clean fruit in storage were unsuccessful, as were also inoculations attempted during picking and packing.

Virus diseases of dahlia. PHILIP BRIERLEY.

Dahlia mosaic is prevalent in New York, New Jersey, Connecticut, and probably throughout the United States. The most characteristic symptom is vein banding. Some dahlia varieties show no appreciable dwarfing or distortion when affected. Others show marked dwarfing, various forms of leaf distortion, or vein necrosis. The mosaic virus from varieties of diverse symptom types produces uniform symptoms in a given variety. The virus has been transmitted by grafting and by *Myzus persicae,* not by mechanical methods nor through seed. The incubation period is usually 4 to 6 weeks.

Dahlia ring spot is characterized by zigzag or ring markings in shades of green, later becoming necrotic. Ring spot has been transmitted, usually together with mosaic, by grafting and is not certainly distinct from mosaic. Yellow ring spot, seen only in stock from Utah, appears as yellow zigzag or ring patterns. Transmitted by grafting, but not by mechanical methods, this virus produces symptoms distinct from mosaic on two varieties. An oak-leaf pattern, apparently of virus origin, is distinct from all the above symptom types on one variety and distinct from mosaic in a second.

A wilt of strawberry caused by Colletotrichum fragariae. A. N. BROOKS.

A sudden wilt of strawberry plants has been observed in the summers 1930–32 in the central Florida area, both in the nurseries and in plants set in the fields. The young leaves of affected plants wilt suddenly, and the crowns show the presence of a reddish brown dry rot in pith and cortex. The rot usually starts in the old runner stub at the base of the crown and progresses in both directions, extending to the bud and portions of the roots near the crown. At higher temperatures the disease kills the plants, while at lower temperatures the progress of the rot is so retarded that the plants are able to survive by putting out adventitious roots above the rotted portion of crown.

The disease has been found associated with anthracnose or runner spot caused by *Colletotrichum fragariae,* and this parasite has been isolated from the centers of rotted crowns and from diseased roots. The ability of this organism to produce the wilt has been proved by artificial inoculations. Incubation periods vary from 2 days at 35° C. to more than 26 days at 15° C.

Relation of the toxin produced by Bacterium tabacum to the pathogenicity and host range of this organism. E. E. CLAYTON.

Bacterium tabacum secretes a powerful exotoxin, which destroys chlorophyl and so causes the characteristic "wildfire" leaf symptoms. This toxin, while comparable in activity to the toxin produced by animal pathogens, differs from them in its properties. It is heat-stable and is not precipitated by mercuric chloride, neutral calcium acetate, or neutral lead acetate. It is not removed from solution by charcoal and passes through Berkefeld, collodium, and cellulose filters. It is not affected by acids but is quickly inactivated by dilute sodium or potassium hydroxide. Leaf-prick inoculations with (a) toxin, (b) toxin and bacteria, and (c) bacteria gave from (a) and (b) typical wildfire symptoms with many species of plants; but from (c) consistent positive results were secured only with Nicotiana species. The toxin inoculations demonstrated that tobacco-leaf tissues are no more susceptible to injury from this agency than tomato or eggplant and distinctly less so than bean or cucumber. The toxin-plus-bacteria inoculations showed that the bacteria will persist for some time in the tissues of all of the above plants from which they can be reisolated and their pathogenicity demonstrated. Field inoculations resulted in heavy infection plus subsequent disease spread in the case of tobacco and occasional lesions without subsequent disease spread in the case of other crops.

A mosaic of the fig in California. IRA J. CONDIT and WM. T. HORNE.

A mosaic-like trouble of fig leaves has long been recognized in California. It may almost be said that every fig tree in the State shows traces of mosaic, though the disease is not generally regarded as commercially important. The meagre literature referring to it is discussed and a description of the symptoms is given. Areas of lighter tissue occur and leaves may be distorted. Both leaves and fruits are affected and in severe cases may drop. Studies are reported on the comparative susceptibility of varieties. Aside from blastophaga, *Blastophaga psenes,* and visitors to the ripe fruit, the insect population on figs is comparatively small. Aphids and thrips are present for brief periods, while the fig mite, *Eriophyes ficii,* is abundant and widely distributed. A mosaic vector has not been found.

Cuttings from mosaic-affected trees propagated in the greenhouse continue to show mosaic. Seedlings from affected plants have remained healthy for about a year in the greenhouse, whereas buds from healthy seedlings set in diseased trees have grown into mosaic-affected shoots. Seedlings several years in the orchard show much mosaic. The trouble under consideration is apparently a true mosaic.

Infection of seed clusters of spinach by Peronospora effusa. HAROLD T. COOK.

The conidia and conidiophores of *Peronospora effusa* were found on the fruit (seed clusters) of spinach seed plants at the Virginia Truck Experiment Station in 1930 and 1932. Sections of the infected fruit showed that the mycelium of the fungus had permeated the pericarp, funiculus, and the integuments of the ovule. The mycelium was not found in either the embryo or perisperm. Oospores were not found in any of the seeds that were sectioned, but they were found in abundance in infected leaves on the same plants. The occurrence of infected seed may afford one means by which *P. effusa* is introduced into new fields or areas.

Pineapple leaf spot. MELVILLE T. COOK.

This disease was described by Larsen of Hawaii in 1910, who attributed it to *Thielaviopsis paradoxa* that entered the plant through punctures made by spines and insects. The disease was most severe in cloudy, moist weather. Similar spots on pineapple leaves in Puerto Rico and other of the West Indies have been attributed to the same cause, apparently without careful study. An outbreak of this disease in Puerto Rico during the past summer was studied by the writer, who failed to find *T. paradoxa* or any other organism in the spots, which were evidently due to weather conditions. The disease develops during wet, cloudy weather and subsides during dry, clear weather. It was more severe on limestone soils than on volcanic soils. Microscopic examination showed the cells in the infected areas black and collapsed. The writer does not question the results obtained in Hawaii, but certainly the fungus is not necessary for the disease in Puerto Rico.

Fusarium basal rot of bulbous iris. DON B. CREAGER.

A Fusarium root and bulb rot of Spanish and Dutch iris was first observed by the writer in the summer of 1929 on Long Island, N. Y. The disease is very destructive to a number of commercial varieties, including Cajanus, Hart Nebrig, and Imperator. The pathogen enters the roots and finally involves the basal plate and scales of the bulb. It is a species of Fusarium of the *Elegans* type closely resembling *Fusarium oxysporum.* Its pathogenicity has been established as a result of inoculations of healthy bulbs in moist chambers and by growing healthy susceptible varieties in artificially infested soil. Preplanting dips with certain fungicides have given fair control in trial tests.

Effect of temperature on brown patch of turf. ARNOLD S. DAHL.

Analysis of five summers' temperature records at the Arlington turf garden, Rosslyn, Va., showed that the occurrence of brown patch caused by *Rhizoctonia solani* varied directly with the temperature. A period of low temperature was not necessary for the fungus to become active. The disease occurred 82 per cent of the days that the minimum temperature was above 21° C. The germination of sclerotia of several strains of *R. solani* obtained from different sources was studied in Petri-dish cultures on potato-dextrose agar at temperatures from 8° C. to 40° C. in 4° intervals. The time interval required for germination at each temperature was recorded. There was some variation between the different strains in response to temperature. Some strains germinated at 8°, others did not, but all germinated at 12°. The shortest period of germination occurred at 28° or 32°, and all strains germinated at 36° but none at 40°. The sclerotia did not require any drop in temperature to facilitate germination and did not germinate so quickly in most cases when the temperature was temporarily lowered as when it was kept constant. Chilling the germinating sclerotia did not increase the amount of mycelial growth except when the cultures were kept at the unfavorable temperature of 36° C. before and after chilling.

Snow mold and brown patch caused by Sclerotium rhizodes. W. H. DAVIS.

Snow mold and brown patch were observed extensively on golf greens, lawns, and meadows in New England. Snow mold generally formed during the thawing of deep snow of long standing; brown patches were caused by the mycelium from germinated snow-mold sclerotia, and a "silver tip" resulted from inoculations of overwintering leaf tips by mycelium associated with snow mold and brown patch.

Specimens of snow mold were received from 54 stations in 15 States distributed in eastern United States as far south as Virginia; west to Idaho; north to Orono, Me.; also, from Ottawa and the Canadian Rockies in Canada.

There were two types of snow mold, the sclerotial and the non sclerotial. This study is limited to the sclerotial type.

Turfs established by planting rhizomes with sclerotia of snow mold attached became infected with brown patch. An average of 175 sclerotia were formed on rhizomes over each square inch of one brown patch. In the field, sclerotia germinated after a rest period varying from 2 to 12 months; under laboratory conditions, when stored dry, 3 months to 2 years; from leaves showing silver tip, 9 months to 4 years, with a maximum viability of 9 years.

The mycelium of the fungus was of two types; nonpathogenic or sclerotia-forming; pathogenic or of sclerotial origin.

Disease-resistant wheats recently introduced from Russia. JAMES G. DICKSON and R. G. SHANDS.

Triticum persicum var. *stramineum* and *T. timophavii,* obtained from the collection of Dr. N. I. Vavilov, have shown resistance to *Puccinia triticina, P. graminis tritici,* and *Erysiphe graminis* and have produced fertile hybrids with the common varieties of *T. vulgare. Triticum persicum* var. *stramineum* has shown greater stability in resistance and varietal characteristics and has proved more suitable agronomically than other varieties tested. *Triticum timophavii* has been immune from rusts in 2 years' field tests and only slightly susceptible to *E. graminis* during the later stages of plant development when the floral structures were slightly mildewed. This species was fairly resistant to scab in 1 year's test. The F_1 generations from crosses of *T. vulgare* with both the Russian species have been intermediate or susceptible to leaf rust. (Cooperative investigations between

the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station).

Nature of resistance of corn to seedling blight. JAMES G. DICKSON, KARL P. LINK, and A. D. DICKSON.

Previous investigations have shown differences in chemical composition generally correlated with maturity of seedling tissues, especially the cortical structures. The rapidly maturing tissues in the resistant seedlings seemingly prevent advance of the fungus and furnish an unfavorable nutritive substrate for the parasite. Embryonic structures in the resistant seedlings have shown a much higher acidity than similar regions in the susceptible plants. The presence of polyuronides, principally polyglucuronides, have been found correlated with the high acidity in these tissues. The resistant seedlings grown at 12°–16° C. contained approximately 20 per cent more uronic acids than susceptible seedlings. Dilute solutions of the uncombined acids gave a pH of 2.5–3.0 and reduced fungus growth when added in low concentrations to the culture solutions. The calcium salts of the acids were much less active in inhibiting fungus growth. (Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture).

Environment and seed treatment in relation to common potato scab. B. J. DIPPENAAR.

The combined relation of soil moisture and soil temperature to the development of common potato scab was studied in the Wisconsin soil-temperature tanks. At each of 5 temperature stages between 13° and 25° C., inclusive, scab development was greatest at 40 per cent of the water-holding capacity of the soil used, successively decreasing at 55, 70, and 85 per cent. Little or no scab developed at 70 per cent of the water-holding capacity or above, while relatively few or no tubers grown at 55 per cent or below were free from scab. Temperature in the same experiments was less important than soil moisture; bad to severe scab developed at each of the temperatures with the same low soil moisture. Both scab and tuber development in these experiments seemed to be best at about 17° C. Simliar results were obtained in experiments in which the temperature fluctuated.

Both 5- and 10-minute treatments of very scabby seed potatoes in acidulated mercuric chloride (02 per cent $HgCL_2$ + 1 per cent HCL) gave as good results against scab as the standard treatments in corrosive sublimate or cold formaldehyde. In the same experiments, however, acidulated mercuric chloride was the most effective against Rhizoctonia.

Perfect stage of Sclerotium gladioli. F. L. DRAYTON.

This fungus was described as the cause of the dry-rot disease of gladioli. Since then, other genera of the Iridaceae have been added to the suspect range, *viz.*, Crocus, Freesia, Tritonia (Montbretia), and Lapeironsia (Anomatheca).

No sexually produced fruiting bodies of this fungus have yet been observed in nature, but the discovery of the sexual function of its microconidia has made it possible to obtain apothecia in artificial cultures as a result of reciprocal crosses between compatible isolates. In these crosses microconidia are applied to receptive structures developed on a stroma.

The apothecia are typical of those found in Sclerotinia. The fungus is, therefore, transferred to this genus under the name *Sclerotinia gladioli* (Massey), n. comb.

Differentiation of green- and yellow-mosaic viruses in tobacco. J. DUFRÉNOY.

Confirming McKinney's results on the complex nature of viruses, it was observed that juice from yellow areas of mosaic-tobacco leaves (as obtained from the field) produced local "yellow" areas where inoculated into mature leaves of tobacco. The cells showed degeneration of plastids, and the cytoplasm, near the nucleus, assumed the pattern of a network of threads, enmeshing a number of small vacuoles where phenolic compounds became abundant.

Leaves developing from upper buds a few weeks after inoculation of lower leaves show typical mosaic (cells showing striated and plate bodies).

The juice from "pale green" areas of mosaic leaves produce no local lesion where inoculated but induce typical green mosaic in leaves subsequently developing from buds.

Nectria coccinea on beech. JOHN EHRLICH.

A menacing disease is resulting in wide-spread mortality to the beech, *Fagus grandifolia,* stands of the Canadian Maritime Provinces. A survey of the affected regions disclosed that approximately 90 per cent of the beeches over 3 in. d.b.h. in forest stands are infected and that approximately 50 per cent are already dead where attack has continued for several years. The disease was caused by *Nectria coccinea,* following attacks by the beech scale, *Cryptococcus fagi.* Field observations, inoculations and other experiments, and histological studies showed that the fungus enters the minutely fissured periderm of trunk and branches where the insect has been feeding and grows rapidly through bark, phloem, cambium, and peripheral sapwood. Death of these tissues and interruption of conduction result. Both fungus and insect are disseminated by wind, the fungal spores during and after rain and the insect during egg and larval stages in summer and autumn. The fungus is advancing southward and westward and has just been found in Maine. The insect already occurs as far south as Boston, Mass. Control lies in protection against or eradication of the insect, which is practicable with ornamental trees, and in the possibility of forest management planned to minimize the severity of attack.

Basal glume rot of barley. CHARLOTTE ELLIOTT and A. G. JOHNSON.

In 1931, typical specimens of basal glume rot of wheat were received from Arkansas. Along with these were specimens of barley heads with some of the kernels showing characteristic, dull brownish black bases, which symptoms suggested basal glume rot. The discolored areas were teeming with bacteria. Isolations from both wheat and barley specimens yielded similar organisms both of which were identified as *Bacterium atrofaciens.*

Inoculations with the organism isolated from wheat reproduced the characteristic symptoms on wheat and barley and from these lesions the typical white organism was reisolated. The organism isolated from barley produced blackening at the bases of spikelets of both wheat and barley, from which pure cultures of the white organism were reisolated. Reisolations of the wheat organism from barley again produced typical discoloration on heads of wheat and barley.

Influence of soil moisture and soil temperature on infection of wheat by Urocystis tritici. J. A. FARIS.

Experiments, in which Harvest Queen wheat was inoculated with flag-smut spores and germinated under controlled conditions of temperature and moisture, indicate that high infection may be secured over a relatively wide range of temperature (10° to 20° C.) when the soil is rather dry during the period of the germination of the wheat.

When the soil moisture was at 40 per cent, that is, at about the point considered ideal for sowing wheat, high infection was secured only when the wheat was germinated at temperatures of 10° and 15° C.

When the soil was almost saturated (60 per cent), high infection was secured at 10° C. Infection percentages dropped very rapidly at temperatures higher or lower than this.

Effect of scab-preventive treatments on apple-tree growth and yield. DONALD FOLSOM.

In a young McIntosh apple orchard in Maine, 350 trees were studied for 5 years in 45 replicated plots. Four series, respectively, received sulphur dry mix, sulphur dust, lead arsenate, and dry lime sulphur during this period and produced average stem diameters of 41, 40, 39, and 38 mm. A fifth series, receiving flotation sulphur 3 years and nothing 2 years, grew to 38 mm. The diameter differences here were not significant statistically and were much less than the generally significant stem-diameter differences found with several natural modifications of the soil, a sandy loam. In an adjacent older McIntosh orchard in 28 comparisons, each involving a series of trees treated with unmodified dry lime sulphur, this material generally tended to increase rather than decrease fruit yield and had no effect on rate of girth increase. Here, in 20 comparisons, using individual-tree data, there generally was no significant correlation between leaf scab, leaf-spray injury, yield, and growth within the same season, while further correlations showed an interpedendence of fast growth, earliness of first yield, and the yield rate for a period of recent years.

Potato tuber rot caused by Botrytis cinerea. DONALD FOLSOM.

Botrytis cinerea was readily isolated in different seasons from a rot of potato tubers stored in Maine. In this type of rot the skin becomes wrinkled, while the flesh easily loses water under pressure. Where advancing rapidly, the rot causes a temporary darkening of the flesh. Different cultures of the fungus, including some isolated from potato foliage and stems during an epidemic of Botrytis blight in the field, have, under certain conditions, caused the same kind of rot as that found in storage. A different form of rot also has been obtained under certain other conditions, where all inoculations have been successful but where the lesion did not penetrate more than 1 or 2 cm. deep. The fungus has been reisolated, reinoculated, and again reisolated, without particular difficulty except where conditions were very favorable for wound repair.

Cytospora canker of Picea spp. C. J. GILGUT and O. C. BOYD.

For many years the dying of lower limbs of blue spruce, *Picea pungens glauca,* and to less extent Norway spruce, *P. excelsa,* has been observed in Massachusetts. In 1923 a limb canker of unknown cause was reported on *P.* sp. from Connecticut. In 1930 a species of Cytospora was found constantly associated with spruce cankers in Massachusetts. The same year, *C.* sp. was reported from New Jersey on *P. pungens kosteri.*

Cytospora limb canker on blue spruce is wide-spread in Massachusetts and frequently involves from ¼ to ½ of the limbs. The disease attacks smaller branches, as well as larger limbs, but trunk infection has not been observed. It usually starts on a lower limb and spreads upward and laterally. There is pronounced gummosis in most cases but rarely, if ever, any hypertrophy or atrophy. No organism other than Cytospora has been found in the cankers.

Spring inoculations with pure cultures of Cytospora from blue spruce produced typical limb cankers by fall, with pycnidial formation, gummosis, and death of limbs, on both blue and Norway spruces. Reisolations yielded the same organism in pure culture.

Method of estimating loss in yield from cereal diseases. F. J. GREANEY.

During the past 8 years, field experiments have been made each season to determine the value of sulphur dusting for the control of cereal rusts. The data accruing from experiments, in which the amounts of disease on individual plots were varied by sulphur dustings at different rates and intervals, were used to estimate the loss in yield due to stem rust, *Puccinia graminis*.

The method employed has been that of correlating rust percentage and yield in a single standard variety. Each year the results were summarized in the form of correlation coefficients and regression equations. When the correlation coefficients obtained were statistically significant it was found that the regression of yield on percentage rust was linear, indicating that uniform increases in rust result in uniform reductions in yield. It was possible, then, to determine the reduction in yield for each 10 per cent of stem rust. The value thus obtained was used in calculating the annual loss from stem rust in Manitoba. The method has been used also to estimate the loss in grain quality from rusts and from some of the minor diseases of cereals.

Production of a white fertile saltant of Helminthosporium sativum by means of ultra-violet radiation. F. J. GREANEY and J. E. MACHACEK.

Preliminary studies on the effect of ultra-violet light on growth and saltation in *Helminthosporium sativum* have shown that some of the strains of this fungus were apparently unaffected by the rays, but the majority produced sector and internal saltants. The saltants, so far, have remained constant. In addition to a decided increase in the frequency of saltation, irradiation produced a marked suppression of growth in those strains affected by the rays.

One strain of *H. sativum* irradiated with ultra-violet light produced a strongly sporulating, albinistic saltant. The spores and mycelium of the saltant are hyaline but, in mass, pale pink. Pathogenicity studies in greenhouse and field have shown it to be as virulent as the parent. It has been recovered unchanged from the diseased plant parts. The problem of fixing the taxonomic position of this new fungus presents some difficulty.

Control of Alternaria dianthi causing a serious disease of the carnation. E. F. GUBA.

Wide differences exist in the reaction of carnation varieties to infection. The disease is of consequence only on plants grown in the field and is occasionally destructive in the cutting beds and in the flats.

Dipping the cuttings in 1–1,000 solution of potassium permanganate for 5 minutes greatly improved rooting and helped to control the disease in the cutting bench.

Laboratory experiments reveal the superior toxicity of Bordeaux 4–4–50 and calcium arsenate 1–50 to the conidia. Bordeaux and calcium arsenate with ½ pt. of linseed oil was the most efficient fungicide in the field. The use of Bordeaux on benched plants in the fall added to the control of the disease.

Benching field-grown plants by the first week in July was the most effective control measure. The age of the plants has an influence upon the prevalence of disease.

Naphthalene fumigation in comparison with syringing with water, for controlling the red-spider mite, indirectly lessened the amount of disease and increased the number of blooms. A spray containing 5 per cent salt and 0.2 per cent nicotine sulphate, which is occasionally used for mite control, was not toxic to the conidia and was shown to increase the hazard from the disease.

Control of the root-knot nematode in greenhouses with carbon disulphide emulsion. E. F. GUBA.

Greenhouse soil treatments with carbon disulphide emulsion have given good control of the root-knot nematode, *Heterodera radicicola,* and have restored normal yields of tomatoes. A stock emulsion containing 68 per cent carbon disulphide, 26 per cent water, and 6 per cent resin-fish-oil soap, diluted in water to make a 1–50 concentration, was applied at the rate of 1 gal. per sq ft. of bed. The cost for materials is approximately 9/10 of 1 ct. per sq. ft. The addition of 2/3 gal. of formaldehyde to the above formula is required to control fungous pathogens and weeds, at an additional cost of approximately 7/10 of 1 ct. per sq. ft.

The removal of the large infested roots when the old vines are cleaned out, a lapse of 2 to 3 weeks to permit decay of root material, and thoroughness of application add to the efficiency of this chemical treatment. A further delay of 1 week after treatment is necessary before planting.

Carbon disulphide emulsion should find particular use in greenhouses not equipped for efficient steam or hot-water sterilization or where it may be more practicable and desirable to use this chemical, and it should encourage a more general use of formaldehyde for controlling fungous pathogens.

Damage to forest products by fungi. CARL HARTLEY.

The financial losses due to the deterioration of forest products are much larger than those caused by equal amounts of deterioration in the living tree. In meeting timber shortage, pathologists can give more immediate help through decreasing the drain due to forest-products fungi than by developing methods for combating diseases of the standing forest. Fungi that cause discoloration are of increasing importance because the sapwood they attack forms an increasing proportion of the lumber we use. New antiseptics, including ethyl mercury compounds and chlorinated phenolates, have been found to give lumber a high degree of protection during the seasoning period at costs for materials little over ½ ct. per 100 sq. ft. of surface protected. Decay is much more important than stain. Various estimates have placed the amount of timber that goes annually to replace destroyed material at between 1/6 and 1/12 of the total cut. Even the lowest estimate, when applied to saw timber, is equal to 41 per cent of the estimated annual growth of such material in the forests of the country. An increasing proportion of the timber in use in exposed situations is protected by preservatives, but recent architectural styles favor decay in frame buildings.

A brooming disease of Robinia pseudoacacia transmitted by grafts. CARL HARTLEY and L. W. R. JACKSON.

The disease is common on sprouts in the Middle Atlantic States. Aside from the conspicuous witches' brooms, no definite symptom has been recognized. Few large trees have been found with visible disease in the tops, and only one showed the burls mentioned in a previous paper. Broom production occurs mainly during late summer. Perhaps because of the abnormally late growth, broomed portions of crowns ordinarily die during the following winter. A root may continue to produce broomed sprouts year after year without signs of abnormal growth in the crown of the tree to which it belongs. An infected tree may to all appearances entirely recover, but trees not noticeably abnormal sometimes send up many broomed sprouts from the trunk after they are cut. It is possible that part of the dying back of the locusts in some localities, without brooming of the crowns, is due to this disease in the roots. A Camarosporium found frequently associated with the disease is not believed concerned in causing it. Graft transmissibility is demonstrated in a paper to appear in an early number of Phytopathology.

Pure-culture technique for quantitative studies of plant growth in association with microorganisms. A. B. HATCH.

An apparatus devised possesses none of the undesirable environmental limitations inherent to ordinary pure-culture methods. It exhibits qualities of environment that are comparable to those that exist in open sand cultures placed in an artificially cooled and dehumidified greenhouse. The plant chamber consists of a copper base, containing a Pyrex jar for sand and supporting an inverted Pyrex jar that is sealed to the base with De Khotinsky cement during sterilization. A continuous stream of sterile air passes into the chamber through a large diatomaceous filter. Sterile nutrient is introduced and removed through a Pyrex tube that extends to the bottom of the sand container end that is bacteriologically sealed at the open end (Doak's technique). The chambers are housed in a small air-cooled compartment of a greenhouse. The culture method permits: (1) complete change of nutrient at frequent intervals; (2) maintenence of an atmosphere of normal composition, humidity, and temperature; (3) direct solar irradiation (through glass); and (4) the growing of tree seedlings in quantity. A wide range of Pyrex jars is available with which chambers may be constructed in sizes suitable for most agricultural crop plants.

True mycorrhizal fungus in contrast to Mycelium radicis atrovirens. A. B. HATCH.

A fungus with black mycelium was isolated from black mycorrhiza of *Pinus sylvestris* growing in Sweden. It is provisionally named *Mycelium radicis nigrostrigosum.* In pure culture with *P. strobus* and *P. resinosa* it formed typical ectotrophic mycorrhiza. Mycelium of the *M. resinosa atrovirens* type, isolated from the same type of mycorrhiza on *P. sylvestris,* overgrew and killed similar pure-culture seedlings. The latter fungus in aerated culture neither attacked the aerial portion of the seedlings nor formed mycorrhiza. It apparently exhibits maximum development on the surface of short roots that have been converted to mycorrhiza by other fungi.

Seed-borne bacteria main cause of pea wilt; Fusarium, Aphanomyces, and other organisms merely subsidiary. LOUISE SOLBERG HEIMBECK.

Pea wilt is best studied on peas grown in water cultures, *i.e.,* Rothamsted solution, as fatty substances then oxidize slowly, so that the cell walls of the roots are easily penetrated by the bacteria, which attack primarily meristematic tissues just becoming differentiated. Root tips are discolored and stop growing. Entrance to the vascular core is chiefly forced through endodermal openings formed by secondary rootlets. The host cells react by coating the intruders with a yellow brown reddish gum-like substance, in which the bacteria lose their identity for a microscopical observer. Stained microtome sections of attacked plants from water cultures, however, disclose the bacteria.

Once inside the translocation system, the bacteria may reach the growing pod and enter the ovule, which, nevertheless, may develop into a viable seed. Seedlings originating from infected seed show vascular discolorations in the cotyledon petioles, in epicotyl, or root. Under good growing conditions they may develop into apparently healthy seed-bearing plants, if cold wet weather or attacks of subsidiary parasites do not so lower their vitality that the bacteria get the upper hand and the plants wilt. Besides existing internally in infected seed, the bacteria live in infested soils.

Increasing the resistance of tobacco ring-spot virus to aging in vitro by use of carbolic acid. R. G. HENDERSON.

In preliminary experiments it has been found that a small quantity of caroblic acid added to expressed juice increases the resistance of the tobacco ring-spot virus to aging.

Virus-infected juice to which sufficient 5 per cent carbolic acid solution was added to make a concentration of 0.25 per cent was highly infectious after incubating for 7 days at 27° C. The virus in juice treated with toluene was not infectious on the seventh day and in untreated juice on the third day.

Water loss in tomato mosaic. J. W. HEUBERGER and J. B. S. NORTON.

Detached mosaic leaves lost 62 per cent of their weight by drying, while healthy leaves lost 39 per cent. Standard cobalt-chloride paper readings on leaves on the plant showed that mosaic leaves were transpiring at a much faster rate than healthy leaves, especially on the upper surface.

Plants grown in nutrient solution showed greater transpiration per gram of dry weight and per leaf-surface area in long-infected mosaic plants than in healthy ones. There was a sharp drop in transpiration coincident with the appearance of the first visible mosaic symptoms, but no certain effect of the virus before that time was observed, as shown by relative water loss or by difference in dry weight. The transpiration rate of the mosaic plants gradually increased after the initial sharp drop. The plants used were selected for equal transpiration, at which time half were inoculated.

Bound water in leaf tissues of heat-resistant and heat-susceptible inbred strains of yellow-dent corn. J. R. HOLBERT and B. C. FRYE.

Total-, free-, and bound-water determinations made by heat-of-fusion-of-ice method, July 17 to August 3, 1932, indicated that water-binding capacity of leaf tissue of heat-resistant strains of yellow-dent corn increased as heat and drought continued, with temperatures ranging from 95° to 103° F. From July 19 to 26, total-water content decreased 5.4 per cent and bound water increased 62.1 per cent (wet basis), or 35.2 per cent. (dry basis). On second day following one-half inch of rain on night of July 25, which resulted in higher relative humidity and lower air temperatures, there was another marked shift in bound-water and free-water equilibria in direction of condition existing prior to heat and drought period; namely, total-water content increased 1 per cent and bound water decreased 23.2 per cent (wet basis), or 21.5 per cent (dry basis).

Water-binding capacity of comparable leaf tissues of heat-susceptible strains increased very little as heat and drought continued and, in some strains decreased significantly. During first 3 days after heat and drought period had passed, heat-susceptible strains made phenomenal growth, and their water-binding capacity increased rapidly and almost equalled that of heat-resistant strains.

Prehardening for heat as well as for cold has been found associated with marked increases in water-binding capacity. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the Illinois Agricultural Experiment Station, and Funk Brothers Seed Company).

Bacterial leaf blight of dent corn. J. R. HOLBERT, CHARLOTTE ELLIOTT and BENJAMIN KOEHLER.

Bacterial leaf blight of corn was prevalent throughout Illinois and other parts of the Corn Belt during 1932, occurring in all of the 66 fields surveyed. By the middle of August some strains and fields presented a general appearance somewhat similar to severe firing from heat and drought. Loss in green-leaf area, directly traceable to local bacterial infection, ranged from a trace to more than 50 per cent, with an average of 16.6 per cent.

Numerous isolations have shown that in most cases the wilt organism present in these leaf lesions was not present in the same leaf veins farther down in the leaf blade nor in

the sheath or bundles of the stalk below the infected leaf. Differences in strains of the pathogen have been noted.

The reduction in leaf area apparently increased susceptibility to Diplodia stalk rot and to chilling and freezing injuires and decreased breaking resistance of stalks and total yield and quality of grain. Similar results were obtained by artificially removing comparable amounts of green-leaf area in plots relatively free from leaf blight.

Different inbred strains and their crosses showed marked differences in resistance to bacterial wilt.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Illinois Agricultural Experiment Station, and Funk Brothers Seed Company).

Differences in size and shape of smut balls of two physiologic forms of Tilletia tritici. C. S. HOLTON.

Observations have shown that 2 physiologic forms of *Tilletia tritici* that attack Hohenheimer wheat may be distinguished by differences in size and shape of their respective smut balls. During the 2 years that these 2 forms have been under observation in the field, 1 of them produced large and somewhat elongate smut balls and the other produced comparatively small and distinctly rounded smut balls. These differences were sufficiently striking and constant to permit positive identification of each of these 2 forms by these means. In addition to these differences, these 2 forms differ also in pathogenicity, prominence of spore-wall reticulation, and, to some extent, in the rate of chlamydospore germination.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington and Oregon Agricultural Experiment Stations).

Inheritance of chlamydospore characteristics in oat-smut fungi. C. S. HOLTON.

The F_1 chlamydospores of crosses between monosporidial lines of *Ustilago avenae* and *U. levis* are echinulate. Approximately 99 per cent of the F_1 sporidia of all the species hybrids studied were non-culturable, and, therefore, a definite segregation ratio for chlamydospore markings could not be determined. However, both echinulate and smooth chlamydospores were segregated in the F_2 generations, which were produced by inoculating with F_1 chlamydospores. The echinulate chlamydospores predominated in all cases.

A cross between two of the F_1 monosporidial lines referred to above produced a buff smut, the chlamydospores of which were smooth and hyaline. The F_1 chlamydospores of crosses between this buff smut fungus and *U. levis* were smooth and brown, and in crosses between the buff smut fungus and *U. avenae* they were echinulate and brown. In the hybrids produced by crossing the buff smut fungus and *U. levis*, the color of chlamydospores segregated in a 3 : 1 ratio. Segregation studies in the hybrids produced by crossing the buff smut fungus and *U. avenae* have not been completed.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.

Influence of root rot on peas. March of the disease. JAMES G. HORSFALL and Z. I. KERTESZ.

An unfolding picture of the incidence of disease on pea roots caused by *Pythium* spp., *Rhizoctonia solani, Ascochyta pinodella, Mycosphaerella pinodes, Fusarium martii*

pisi, and other microorganisms, and the influence of this disease complex on the morbid physiology of the plants was obtained by collecting 50 random plants weekly during the 1932 season from an area artificially infested with heavily contaminated field soil and from a contiguous area of normal soil. Trend curves show how the incidence of disease was correlated with temperature and rainfall and how it stunted the plants; how it decreased the relative and absolute amounts of dry matter per plant; why this decrease was due to disease alone and not to low fertility or soil toxins in the infested soil; how the disease cut short the blooming period, resulting in fewer pods and peas per plant, although the number of blossoms or pods was a function of the dry weight per plant (1 pod per 0–90 gm. dry matter) irrespective of disease; how root rot forced a larger proportion of the total dry material in each plant into the peas themselves; and how it curtailed by 20 per cent the yield of "fancy" grade peas per unit area.

Sclerotium diseases of grains and grasses. C. W. HUNGERFORD and RUTH REMSBERG.

Diseases of grains and grasses produced by several sclerotial fungi that appear in the field after the snow melts have been increasing in occurrence and importance in the high-altitude regions of Idaho during the last few years. Fourteen cultures have been obtained of these organisms, whose optimum temperatures for growth range from 0° to 10° C. These 14 fungi have been placed in 4 groups, according to their morphological and physiological characteristics. One of these groups corresponds in growth characteristics to a culture of *Typhula graminum*, supplied by H. Tasugi of Japan. Attempts made to obtain the perfect stage of these organisms have not been successful. Pathogenicity of several of the organisms has been established by both laboratory and field inoculations. The disease apparently caused by *T. graminum* is best controlled by late seeding and also by using resistant varieties of cereals.

Zinc hydroxide, a substitute for calcium hydroxide in arsenical sprays. R. H. HURT.

Zinc hydroxide seems to be a promising substitute for calcium hydroxide for the prevention of arsenical injury to the foliage of peach trees and snap beans. This material, in combination with arsenicals, may be used either as a dust or as a liquid spray. In either case the zinc hydroxide should replace the hydrated lime pound for pound. For the prevention of arsenical injury in large commercial spraying operations, it will no doubt be more economical to use the zinc-lime combination (8 lb. zinc sulphate, 8 lb. hydrated lime, and 100 gal. water) that is now in general use in Virginia as a peach spray. But, for small-scale operations, zinc hydroxide in combination with arsenicals, should prove economical and effective when used as either a dust or spray. Present results show that certain varieties of apples are subject to russeting by zinc hydroxide. Such injury, however, does not seem to occur when the zinc hydroxide is used in combination with sulphur. Zinc hydroxide may be prepared in the powdered form and then used or stored in the same way as hydrated lime.

Peach mosaic. LEE M. HUTCHINS.

A new virosis of the peach has been discovered in Texas. Known distribution is confined to a few counties in the central and in the southern portions of the State. The disease is spreading, a few home orchards are severely infected, but occurrence is as yet rare in commercial orchards. The disease is communicable by budding and by root grafting. Outstanding characters are: striking mosaic patterns in young leaves, an abundance of small, crinkly leaves with wavy margins, short internodes, clusters of twigs that somewhat resemble witches'-brooms, and irregular, "bumpy" fruits. The mosaic character of the leaves may be fugitive, and the twigs behave variously in growth characters.

Bacterial wilt of corn. S. S. IVANOFF.

An underground agent of transmission of Stewart's wilt disease of corn was found to be the western corn root worm, the larval stage of *Diabrotica longicornis*. Such larvae, which had fed upon the roots of plants infected with *Aplanobacter stewarti*, repeatedly transmitted the disease when placed on the roots of healthy plants in the greenhouse. In addition to the typical Stewart's wilt organism several bacterial cultures were isolated from wilted plants from various localities and were purified by single-cell isolations. Following their inoculation into corn, there appeared certain symptoms, such as leaf stripes, stalk rot, yellowing, and wilting, which have frequently been associated with Stewart's wilt. Field inoculation trials with these bacteria on yellow-dent and sweet corn showed differences both in the severity of disease in individual plants and in the percentage of disease in the inoculated plants. Bacteriological studies thus far have shown that these cultures differed consistently both from the typical Stewart's wilt organism and from one another in colony characters and in certain physiological reactions.

Effect of sulphuric acid and aluminum sulphate, as used for the control of damping-off of conifers, on soil pH. L. W. R. JACKSON.

pH determinations made in the seed beds at a number of nurseries throughout the United States show that the reaction change is restricted to the upper soil layer. For most of the soils tested, the surface application of either sulphuric acid or aluminum sulphate did not change the pH much below a depth of 2 in. The maximum reaction change, as shown by the lowest pH, usually occurs at a depth of ½ in. The degree of acidification at the time of treatment depends on the nature of the soil.

Effects of H-ion and aluminum-ion concentrations on conifer damping-off. L. W. R. JACKSON.

Seedlings of *Pseudotsuga taxifolia* and *Pinus ponderosa* were grown and inoculated with Pythium and Rhizoctonia in liquid and quartz-sand cultures. The pH varied with intervals of 1 unit from 2.5 to 8.5. A nutrient solution, stable through a wide pH range in the presence of iron and aluminum, was developed by using disodium glycerophosphate as a phosphate source. In liquid cultures without aluminum, damping-off percentages increased from 0 at pH 2.5 to a maximum near the neutral point. In sand cultures damping-off percentages also increased with the pH. In sand cultures the addition of aluminum-ions by means of aluminum sulphate appeared to cause further decrease in damping-off at pH 3.5 but not at pH 6.5. pH 2.5 was always toxic to seedlings, while at pH 3.5 growth was excellent. Fungi were grown in the same solution and at the same pH. Growth of several Pythium cultures from both acid and alkaline soils was inhibited at pH 2.5 and 3.5 while it increased from pH 4.5 to 6.5, and then decreased to almost complete inhibition at pH 8.5. There was also a relationship between pH and chlamydospore and oospore production. Rhizoctonia was able to grow over a wider range of acidity, making appreciable growth at pH 3.5.

Physiologic forms of Bremia lactucae on lettuce. IVAN C. JAGGER and NORMAN CHANDLER.

In breeding lettuce for resistance to mildew (*Bremia lactucae*), several apparently distinct physiologic forms of the fungus have been encountered. In trials at Sanford, Fla., in 1922, 9 varieties of lettuce showed a high degree of resistance. The same varieties showed similar resistance at Chula Vista, Calif., during succeeding seasons. In 1926 only 2 of these varieties showed marked resistance in England to the apparently

distinct physiologic form of the fungus encountered there. In 1927 3 varieties were highly resistant to a third physiologic form prevalent that season in Imperial Valley, Calif.

The variety *Romaine blonde lente à monter*, obtained from France, has, so far, shown considerable resistance to all forms of the fungus encountered. Crosses with this have given several strains of New York or "Iceberg" type lettuce that are highly resistant to mildew at Chula Vista. In central California there seems to be a fourth physiologic form of mildew, which attacks several of these strains in various degrees, but, so far, the strain "Imperial D" has proved practically immune. Recent parallel inoculations with these two physiologic forms of *Bremia lactucae* have demonstrated marked differences in their ability to attack several strains of lettuce under similar conditions.

Nature of injury caused by potato leaf hopper on forage legumes. HOWARD W. JOHNSON.

Artificial inoculation with juice from yellowed and reddened leaves and from adults and nymphs of *Empoasca fabae* failed to produce symptoms of the injury, nor did symptoms develop on plants started as shoot cuttings from yellowed and reddened plants. Confining an adult *E. fabae* upon restricted areas of the leaf petioles of Ladino clover for 8, 16, and 24 hours resulted in the redding and yellowing of about 20, 60, and 50 per cent of the leaves, respectively. With the 24-hour feeding exposure, an additional 40 per cent wilted rapidly and turned brown. As compared with green leaves from non-infested plants, the yellowed and reddened leaves of alfalfa and Ladino clover are higher in dry matter, osmotc presesure of cell sap, reducing sugars, sucrose, starch, total polysaccharides, and alcohol-soluble nitrogen but are lower in total nitrogen. Symptoms almost identical with those caused by the feeding of this leaf hopper were produced artificially by mechanically severing all but one of the vascular bundles in Ladino clover petioles and by girdling Ladino petioles and alfalfa stems with live steam. The chemical changes in leaves reddened and yellowed by artificial girdling and by leaf hoppers were essentially the same. These studies indicate that the reddening and yellowing caused by this leaf hopper are not the result of the injection of a specific inciting agent; virus, or toxin but are caused by an overaccumulation of the carbohydrate products of photosynthesis, especially the osmotically active reducing sugars.

Cucumber mosaic on tobacco in Wisconsin. JAMES JOHNSON.

Effect of leaf-rust infection on water economy and growth of two wheat varieties. C. O. JOHNSON and E. C. MILLER.

Two varieties of spring wheat, one susceptible and the other resistant to leaf rust, were grown in sealed containers in the greenhouse and the water requirement determined for plants of various groups, arranged according to the time of initial leaf-rust infection.

Following is the amount of water, in grams, required by each group of the varieties Pusa No. 4 (susceptible) and Warden (C. I. 4994) (resistant), respectively: 905.3 and 500.8, inoculated first in seedling stage; 869.9 and 490.0, inoculated first in jointing stage; 791.4 and 509.3, inoculated first in boot stage; 619.1 and 480.7, inoculated first when fully headed; 456.8 and 510.8, non-inoculated controls.

In the susceptible variety, the water requirement increased with increasing length of association of host and parasite. This was due principally to the depression of such plant characters as height, leafiness, tillering, and size of stems. Root development, date of heading, and date of maturity also were much retarded in infected plants of the

susceptible variety. These retardations were less marked in the resistant variety. The height of inoculated plants of the resistant variety was slightly reduced and the time required to mature slightly increased, but the yields of tops, grain, and roots were not materially affected.

Two stem cankers of the China aster. L. R. JONES and REGINA S. RIKER.

Two diseases of the China aster were observed in Wisconsin and Illinois, September, 1932, characterized by cankering of the aster stem that may lead to wilt. Each disease showed distinctive symptoms, but there was enough similarity at certain stages to permit confusion with the familiar Fusarium wilt. The commoner and more serious of these cankers was caused by *Phomopsis callistephi,* described as a new species from Illinois in 1925 by Tehon and Daniels. This disease, as seen in September, differed in general from the Fusarium wilt in that (1) it seemed cortical rather than vascular; (2) it developed as a lateral canker on the aboveground stem rather than from the roots as a basal stem rot; (3) it showed abundant pycnidia on the diseased stem surface in the later stages. Phomopsis occurred in 4 out of 5 cloth-house aster plantations visited in Wisconsin and Illinois. In the worst case, where asters had been longest in culture, more than half of the aster plants of some strains were attacked. The other stem canker, caused by *Botrytis* sp.. was observed twice, in both cases associated with excessive moisture. It was of minor importance and relatively easily identified.

Seed transmission of the virus causing variegation of Abutilon. JOHN Y. KEUR.

The virus that produces variegation in *Abutilon thompsonii* and *A. mulleri* is in a limited way transmissible to some of the seedlings obtained by crossing these 2 clons. Out of 536 seedlings obtained from this cross 287 were green and 249 showed "variegated" characteristics, which could be transmitted in but 4 instances to susceptible green *A. reguellii* scions by grafting.

Effect of carbon dioxide on cultivated mushrooms. E. B. LAMBERT.

By running carbon dioxide under bell jars containing growing cultivated mushrooms, it was shown that the accumulation of carbon dioxide in the air caused abnormal growth, stunting, and even death. Further experiments indicated that these effects were caused by carbon dioxide and not by the dilution of oxygen. It also has been demonstrated that the harmful effects were due principally to the carbon dioxide surrounding the sporophores rather than to that in the interstices of the compost in the bed. Approximately 1 per cent carbon dioxide was the lowest noticeably injurious concentration.

Life-history, morphology, and cytology of Polymyxa graminis. G. A. LEDINGHAM.

This obligate parasitic fungus was discovered at Toronto in 1930 in the roots of Marquis wheat seedlings grown in Ontario soil. Two distinct types of thalli, one consisting of thin-walled zoosporangia and the other of resting sori, are found in the life cycle.

The large, irregular septate zoosporangia are surrounded by a definite membrane and liberate numerous biciliate zoospores through exit tubes.

The resting sori are round to elongate and several are often arranged linearly in the host cell. These multinucleate thalli segment to form spore balls.

Two types of nuclear division occur during the development of both the zoosporangia and the resting spores. Protomitosis occurs in the early divisions concerned with the growth of the thallus. Mitosis is found when segmentation commences.

The character of the resting sori and the occurrence of protomitosis in the growth of the thalli indicate relationship with the Plasmodiophorales. Because of its well-developed zoosporangia, unique among members of this order and showing affinities with the Chytridiales, this fungus is worthy of recognition as a new genus to which the name Polymyxa has been given.

Bacterial twig and blossom blight of raspberry. S. G. LEHMAN.

In June, 1932, the writer received some young diseased raspberry shoots bearing flower clusters with open blossoms and half-grown berries. The stems of the flowers and berries had died back for distances of 1 to 4 in., presenting the general aspect of fire blight (*Bacillus amylovorus*) resulting from blossom infection on apple. The diseased tissues were light brown and showed numerous small white droplets of bacterial ooze. Some of the leaves showed marginal dead areas bordered by brown water-soaked tissue that contained bacteria.

A bacterial organism isolated from the diseased flower stems was found to be pathogenic when inoculated into raspberry cane tips. It has been recovered from artificially inoculated twigs and used in successful reinoculations. This bacterium is also pathogenic on wild blackberry. It has thus far given only negative results when inoculated into apple water sprouts. Inoculations onto raspberry with bacteria isolated from apple fire-blight twigs and thought, but not proved, to be *B. amylovorus* have given negative results. Apparently, no one has published proof that raspberry is a suscept to *B. amylovorus*. Further studies will be necessary to a positive statement as to the relation of the raspberry-blight-producing organisms to *B. amylovorus*.

Crown gall on Sahuaro (Carnegiea gigantea). MICHAEL LEVINE.

Crown gall is unknown in nature in the Cactaceae. The production of well-defined crown galls on Sahuaro, the tree cactus, is reported for the first time. The galls were induced by inoculation of plants 5 to 6 years of age with a virulent strain of *Bacterium tumefaciens*. The growths appear slowly at first and then develop more rapidly as the tumor becomes macroscopically recognizable. Tumors, 1 year after inoculation, are still very young. They section readily and present characteristic structures comparable to those on the beet.

The cells of the crown-gall tissue show an abundance of starch but in no case are mucilage or slime cells observed, although the host tissue in close proximity to the tumor tissue appears to have many more mucilage cells than are found in more distant parts of the plant.

A number of species of Opuntia also were inoculated. Well-rooted plants, as well as parts of joints kept in sterile Petri dishes, failed to respond to inoculations with *Bacterium tumefaciens*.

Elm diseases in America. O. N. LIMING.

The Dutch elm disease is now wide-spread in Europe. Eight cases were found in America and eradicated in 1930 and 1931. No cases were discovered in 1932. Scouting has been limited and other infection centers may exist. In the three seasons specimens from 1,407 suspected trees were cultured at the Dutch Elm Disease Laboratory at Wooster, Ohio. Of these, 1,045 showed parasitic fungi, of which 146 were Verticillium and 336 a fungus referred to Cephalosporium. Trees showing Verticillium one year seem to recover the next, but it is not yet known whether this recovery is permanent; trees affected with Cephalosporium show an increase in the diseased condition the second year. A root disease in southern Ohio, Indiana, Illinois, near-by Kentucky, and West Virginia is a complicating factor in the elm-disease work.

Relation of mechanical injury to foot rot of cereals. J. E. MACHACEK and F. J. GREANEY.

Preliminary greenhouse trials have shown that when injured seed of several varieties of wheat and of hulless oats and barley is planted in soil infested with *Fusarium culmorum* var. *letius*, the damage from foot rot and preemergence blight to the resulting seedlings is greater than when uninjured seed of the same varieties is sown. The germination of wheat seed injured by clipping off a portion of the brush end or by scarifying the surface with sandpaper is only slightly reduced, but the amount of disease is markedly increased.

These results were confirmed by carefully planned field experiments in which scarified and uninjured seed of Marquis and Mindum wheat was planted in infested soil. The "analysis of variance" method was used in analyzing the field data. In 1932 a significant increase in disease rating and a significant decrease in yield were obtained through seed injury. When the disease ratings and yields of individual plots in the field experiments were correlated, a highly significant negative correlation coefficient was obtained.

Effect of pressure and amounts of copper applied in spraying potatoes. E. O. MADER.

A 4-year series of potato-spraying experiments to determine the influence on yield of pressures and amounts of copper applied has given consistently the following results. All sprayed plots outyielded the unsprayed. Using nozzles with a small orifice, size 1.18 mm., the amount applied and the yield per acre increased with each increase in pressure up to 600 lbs. Greater increases in yield were obtained by increasing the pressure from 200 to 400 lbs. than by increasing it from 400 to 600, illustrating the law of diminishing returns. With larger nozzle orifices, giving larger amounts of spray per acre, no increase in yield was secured beyond 400 lbs. Here, also, the law of diminishing returns appears to operate. The increase in yield, with large nozzle orifices over small, was greatest at 200 lbs. pressure, less at 400 lbs., while at 600 lbs. pressure there was a slight decrease in yield. These increases in yield are due to the better distribution of the spray at higher pressures and to the application of larger amounts of copper.

Evidence of stimulation of potatoes by Bordeaux mixture. E. O. MADER.

Four years of experimental work affords direct evidence of copper stimulation in sprayed and dusted potatoes. With moderate infestations of leaf pests and absence of blight, the greater the amount of copper applied during the season and the better the distribution (higher pressure) the greater the number of leaflets per plant, the higher the total green weight of tops, and the longer the plants remain green. Despite this increase in top growth, the largest yields and most uniform tubers were obtained with applications totaling 70–80 lbs. of copper sulphate per acre for the season. Stimulating top growth by applying the greater part of the copper during the fore part of the season gave a significant increase in yield of about 30 per acre over plots in which the greater part of the copper was applied during the latter part of the season. Analyses for copper show that the amounts stored in tubers from sprayed or dusted plots increased with the amounts of copper sulphate applied during the season. More copper is found in tubers from plots receiving increasing concentrations than in those receiving decreasing concentrations.

Disease of cultivated sweet violet caused by Sphaceloma. L. M. MASSEY, R. P. WHITE, and A. E. JENKINS.

The disease affecting outdoor and greenhouse violets, in one case identified possibly as edema, were severe in New York in 1929, and in Pennsylvania in 1932. It was

found in Georgia in 1925–1927. Lesions may be numerous, almost covering the plant. Stems, peduncles, and petioles are sometimes noticeably swollen and leaves distorted. Lesions are generally whitish to vinaceous buff; on stems, peduncles, and petioles they are linear to elongate, 5 ± mm. in length, raised or depressed; on leaves generally circular or nearly so, commonly visible at first on lower surfaces as minute water-logged areas, enlarging to at least 3 mm. across, often raised on one leaf surface and depressed on the other.

The Sphaceloma causing the disease is apparently undescribed, at least in this genus. Conidiophores are typically hyaline, soon becoming colored, 0 to 1-septate, 9–12 $\mu \times 2$–4 μ, pointed or obtuse; the conidia are spherical to oblong-elliptical, of various sizes, often 2–3 $\times$ 3–7 μ; hyaline or colored. Three-week-old cultures on slants of potato-dextrose agar were raised, wrinkled, about 1 cm. across, and so brown as to appear nearly black, bordered by dark olive buff; corresponding cultures on cornmeal were nearly smooth, appressed, and ochre red. A more complete description, with a name, will be published later.

Form of toxicity surface for copper sulphate and for sulphur in relation to conidia of Sclerotinia americana. S. E. A. MCCALLAN and FRANK WILCOXON.

The effect of a toxic agent on the germination of fungus spores is best shown quantitatively by means of the *toxicity surface*, a solid model showing the relation between germination percentage, concentration of toxic agent, and time. Two such models are exhibited for copper sulphate and for sulphur dust. It is pointed out that such models enable the investigator to compare the relative accuracy of the different methods of performing toxicity experiments, since the accuracy of a determination at any point is related to the germination percentage obtained and to the slope of the toxicity surface at that point. The form of these surfaces might also lead to certain deductions regarding the mechanism of toxic action.

Spores in the upper air. F. C. MEIER, J. A. STEVENSON, and VERA K. CHARLES.

In the course of efforts to obtain additional information concerning fungus spore content of upper air currents, movement of such spores and their precipitation and viability after exposure at high altitudes, agar-plate collections made in 1932 on 5 flights over the eastern United States netted cultures of some 50 organisms. Flights by the senior writer on routine trips of the U. S. S. Los Angeles and by airplanes were made possible by cooperation with the Navy, Army, and Coast Guard. Collections made at altitudes of 500 to 18,000 ft. indicate the strong possibility of certain types of spores of fungi that cause plant diseases travelling long distances in viable condition before falling to start new local infections.

Spore content at different altitudes varied with atmospheric conditions during and preceding the flight with usually a decided thinning out above 8,000 ft. Viable spores of Pestalozzia were collected March 22, 18,000 ft. above Washington in a strong west wind. Fungi of the following genera have been identified, Acremoniella, Alternaria (Macrosporium), Aspergillus, Chaetomium, Cladosporium, Coniothyrium, Dematium, Epicoccum, Fumago, Fusarium, Helminthosporium, Penicillium, Pestalozzia, Sclerotinia, Stachybotrys, Stemphyllium, and Trichoderma.

A Pythium disease of turf. JOHN MONTEITH, JR.

Pythium was isolated in 1926 from diseased creeping-bent turf at the Arlington turf garden, Rosslyn, Va. The species was identified by Drechsler as *Pythium butleri*. Inoculation in the greenhouse proved the fungus to be a vigorous parasite on various grasses, included species of Agrostis and Poa. Affected patches, usually ½ in. to 4 in. in

diameter, first appear blackened and water-soaked but soon shrivel and turn brown. These patches commonly occur in groups, frequently in streaks, apparently due to distribution of spores by water washing over the surface or on mowers or the shoes of persons walking on the turf. Patches often coalesce and destroy large areas of turf. The disease has been identified on golf courses and lawns in the vicinity of Washington, D. C., Philadelphia, St. Louis, and Chicago. It has been reported in many other sections. The disease is most destructive in periods of high temperature accompanied by abundant moisture. It caused extreme damage to turf during 1931. Inoculations showed the fungus capable of attacking plants over a wide temperature range, with an optimum of 35° C. Since abundant moisture is required for development of this disease it may be checked by judicious watering of turf. No satisfactory fungicidal treatment has been developed, although spraying with solutions of corrosive sublimate has shown some promise.

An undescribed sclerotium fungus prevalent in northeast Texas. D. C. NEAL and R. E. WESTER.

A fungus resembling *Phymatotrichum omnivorum* was discovered at Greenville, Texas, in September, 1932, growing on decayed cotton stalks and roots a few inches below the surface of the soil, following a period of wet weather. Sclerotia formed in abundance as elongate swellings along the mycelial strands similar to the cotton-root-rot fungus. They were white to pale yellow,, depending on age, and varied considerably in size and shape. They averaged about 1×5 mm., were round to ovoid, ellipsoid, variously constricted, and often forked at the strand connections. The sclerotia were smooth and formed of anastomosed hyaline hyphae.

Features that distinguish the fungus from *Phymatotrichum omnivorum* are the finer texture of the strands and sclerotium formation on agar. The mycelium is septate and branching is either opposite or alternate just below the septa. A characteristic behavior of certain new cells arising from the side of a parent hypha is to grow in opposite directions. In some cases the lower portion of the cell grows downward for only a short distance, while the upper part continues growth and forms new cells either parallel, or at an angle of 45°, to the original hyphae. Pending further studies, the name **Ozonium texanum**, sp. nov., is proposed.

Morphology and life history of the cotton-root-rot fungus in Texas. D. C. NEAL, R. E. WESTER, and K. C. GUNN.

Comparisons of the structure of the strand hyphae and sclerotia of *Phymatotrichum omnivorum* have shown that some strands are, in reality, elongated sclerotia comparable to the rhizomorphs of certain basidiomycetes and function not only to spread the fungus vegetatively but also to maintain it in a viable condition during periods of inactivity—a point that was recognized by one investigator as early as 1916. Strands obtained from old cotton roots in fallow plots from areas planted in nonsusceptible crops and from soil cultures several months old were viable in germination tests. The importance of the strand hyphae in the overwintering of the disease in the soil was further demonstrated by pure-culture isolations from some of these strands and by infection experiments.

Sclerotia occurred more abundantly in primary centers of infection following prolonged grain rotation or fallow periods than in plots planted continuously to cotton. The gross morphology and cellular structure of some of these sclerotia indicate that they had formed in the soil at the time of the last cotton planting and had remained in a viable condition during the intervening period of grain rotation or fallow treatment.

Breeding Fusarium-yellows-resistant celery. RAY NELSON and L. C. COCHRAN.

For the past 5 years yellows (*Fusarium* sp.) has become increasingly serious in the commercial varieties of easy-blanching celery, and, in 1931, under especially favorable conditions, the disease caused a loss of $250,000 to Michigan growers. The tall strain of Golden Self Blanching, the leading market variety, is extremely susceptible to yellows. The so-called short-strain varieties, like Golden Plume, are moderately resistant, but under favorable conditions for the disease they produce unsatisfactory crops.

A number of selections were made from fields of the tall strain in 1930, but only one plant was carried through the winter to seed production. The F_1 and F_2 progenies of this plant have shown high resistance when tested in greenhouse and field under the most favorable conditions for yellows. The plants of this variety ("Michigan Golden") are intermediate in type between the tall and short strains, and field tests have indicated that they possess satisfactory commercial qualities. Seed in sufficient quantity for field testing in 1933 is now available.

Other selections, made in 1931, from both the tall and short strains are being tested and reselected. Some of these selections have shown considerable resistance to yellows, but further improvement is necessary before they can be considered satisfactory for commercial use.

Influence of pollen and ovule infection in seed transmission of bean mosaic. RAY NELSON and E. E. DOWN.

It is known that bean mosaic is transmitted irregularly through the seed and that not more than one-half of the seeds are infected in pods harvested from plants arising from diseased seed. To test the possibility of pollen infection and to determine the relative effects of pollen and ovule infection in seed transmission of the disease, crosses were made between Refugee and Early Prolific, varieties very susceptible to mosaic.

The incidence of mosaic in hybrids between mosaic Refugee and healthy Early Prolific was approximately the same as in hybrids between healthy Refugee and mosaic Early Prolific. The mosaic parent in all crosses was produced from infected seed. The results indicate that about ¼ of the ovules and pollen grains are infected with virus.

In crosses between Refugee and Robust, a highly resistant variety, about ¼ of the hybrid seedlings were severely affected with mosaic.

Studies on the resistance of wheat and oats to stem rust. MARGARET NEWTON and A. M. BROWN.

A preliminary study of the nature of resistance in wheat and oat plants to stem rust, *Puccinia graminis*, has shown that, at least in some varieties, meristematic and newly differentiated tissue produces an entirely different infection type from older tissues. In the adult stage, Hope and H–44–24 are highly resistant and Pentad and Acme moderately resistant to all physiologic forms of wheat stem rust, *P. graminis tritici;* yet, when, by injection through the sheath, the spores are brought into contact with newly formed tissue, pustules of a susceptible type are produced. Oat plants, when inoculated in the same manner with forms 21 and 36 of wheat stem rust, develop infection areas with pustules of types 2, 3, and even 4.

Stripe rust in Canada. MARGARET NEWTON, T. JOHNSON, and A. M. BROWN.

Puccinia glumarum tritici has been collected in Canada as far east as 102° W. longitude, or a little beyond the eastern limit reported for this rust in the United States. Two physiologic forms have been found in Canada; at Duncan, British Columbia, a virulent one, which attacks most of the common and durum varieties, and at Olds, Alberta,

a much less virulent one. Both of these forms have been isolated from material sent in from the United States. The less virulent form does not appear to be present in Europe, but the more virulent form is similar to one of the well-known forms in Germany (Giessen).

Freshly formed urediniospores retained their viability and were capable of producing good infection after storage for well over 3 months at 5° C. and a relative humidity of 50 per cent. Some spores germinated at the end of 128 days, the best germination occurring at 10° to 12° C.

The reactions of wheat varieties to strip rust were profoundly influenced by temperature. At 78° C. all the hosts tested with the different physiologic forms were completely resistant, while at 54° C. many were completely susceptible. A number of wheat varieties susceptible in the seedling stage have been found to develop considerable resistance in later stages of growth.

Puccinia glumarum hordei has not been isolated so far in Canada. All isolations from barley varieties have proved to be one or other of the above-mentioned *tritici* forms.

Factors influencing type and sequence of tomato-mosaic leaf abnormalities. J. B. S. NORTON and J. W. HEUBERGER.

Types of leaf abnormalities noted in tomato mosaic were filiform-leaf, cut-leaf, fern-leaf, and mottling. Ordinarily tobacco-mosaic virus, as well as the tobacco-mosaic-virus complex (2 or more tobacco viruses) present in Maryland fields, induced the more extreme leaf abnormalities in young tomato plants during periods of low-light intensity and short-day length. Parallel series with cucumber-mosaic virus produced only mottling under the same conditions. Filiform-leaf usually appeared first, followed by cut-leaf and fern-leaf, then mottling only. Under longer-day conditions mottling may be the only effect after the primary bunching of the young leaves is seen. The production of leaf abnormalities appears to be correlated to a greater degree with low-light intensity than with short-day length.

Germinating seeds and seedlings could not be infected prior to the appearance of green color.

Reaction of tissues from individual plants to soft-rot bacteria. C. R. ORTON.

A pathogenic strain of the *Bacillus carotovorus* type failed to rot certain sterile carrot blocks cut from stored roots. Differences in susceptibility of individual plants were suspected and it was found in duplicated tests on individual cabbage heads that 2 out of 10 or 20 per cent of stored cabbages were very highly resistant to rot when inoculated with *B. phytophthorus* or *B. aroideae,* both strains reacting in approximately the same manner. Under comparable conditions of high humidity the midveins of cabbage were about 15 per cent more susceptible than the laminae of cabbage leaves taken near the midvein.

Tests of 4 varieties of carrots taken from the soil at maturity and split lengthwise show 100 per cent susceptibility to both strains of soft-rot bacteria but, after storage for 3 months, showed only 73 per cent susceptibility to *Bacillus aroideae* and 53 per cent susceptibility to *B. phytophthorus.* Similar results were obtained with inoculations on stored turnips in which approximately 70 per cent of the individual roots rotted when inoculated with either strain.

It is apparent that carrot roots that are susceptible to bacterial soft-rot at growth maturity may undergo changes in storage that induce resistance in certain roots and that laboratory and classroom tests of cultures should be conducted on at least 10 individual roots or plants to assure one that the organism is pathogenic.

Serum-agglutination studies with soft-rot bacteria. C. R. ORTON and ALFRED R. STANLEY.

Recent work in this country and in England has led some investigators to the conclusion that *Bacillus aroideae, B. carotovorus, B. phytophthorus,* and other bacteria causing soft rots in vegetables can be distinguished serologically and, on this basis, are to be regarded as separate species.

Studies with 50 isolants of bacteria of this group show that these organisms are not to be specifically distinguished by agglutination tests. For instance, 2 cultures of *Bacterium dissolvens* isolated from stalk rot from 2 varieties of sweet corn are identical in routine physiological tests but show no sign of cross agglutination. However, when using an antiserum from one of these cultures, cross agglutination is obtained with *Bacillus coli communis* and a *B. aroideae* from Japan isolated from Pai Ts'ai.

Likewise 2 cultures of *Bacterium tabacum* from Pennsylvania were agglutinated by *Bacillus aroideae* antiserum to as great an extent in the case of 1 culture and to a greater extent with the other culture than was a culture of *B. aroideae* from Japan.

It is evident that these numerous soft-rot bacteria, as well as such leaf-spotting forms as *Bacterium tabacum,* are not to be considered as distinct species but rather as variants of the *Bacillus coli* group.

A bacterial blight of the broad bean in Louisiana. L. H. PERSON.

In the spring of 1932, fields of the broad bean in Louisiana were severely attacked by a blight. The leaves were covered with circular, dark brown to black spots, varying in diameter from 2 to 5 mm. Usually, the spots would coalesce and the whole leaf would become black and curled and finally die. A bacterium isolated from these spots produced, when inoculated into the stems of healthy plants, a typical streak disease. Streaks extended for ½ to 1 in. beyond the inoculation punctures. In a number of cases the stems were entirely dead in the vicinity of the puncture after 3 to 5 days. Inoculations made by painting the leaves with a water suspension of the bacteria produced typical dark, circular areas, similar to those observed in the field. The disease has been observed in Louisiana for many years, but this is apparently the first report of its occurrence in the United States. Whether it is the same bacterial disease that occurs in Europe has not yet been determined.

Dissemination of fire-blight bacteria by honeybees. A. L. PIERSTORFF and HOWARD LAMB.

In an effort to gain additional information on the dissemination of the fire-blight organism, *Bacillus amylovorus,* by the honeybee, 2 Yellow Transparent trees were enclosed with cheesecloth. A hive of bees that previously had been infested with fire-blight bacteria was placed beneath one tree, just as the blossoms were opening. The fire-blight organisms did not infect the blossoms within this cage as a result of bee visitations.

On the lower branches of another tree, similarly caged, 19 blossom clusters were inoculated by atomizing with infested bouillon when the center blossom opened. Approximately two thirds of the blossom clusters on this tree were blighted by the bacteria being disseminated from the inoculated blossoms by a noninfested hive of bees. Rainfall did not occur during this interim, so that insect dissemination is the only logical conclusion.

After remaining beneath these caged trees for 5 days, both hives were transported to another location and 1 placed beneath each of 2 similarly caged Grimes Golden trees. The blossoms did not become blighted as a result of bee visitations.

The first part of the experiment was repeated in a location farther north that had a later blooming date. Comparable results were obtained.

Combating spinach damping-off by seed treatment. P. P. PIRONE.

Great losses to spinach from damping-off, caused chiefly by a Pythium, occur on Long Island. Following Horsfall's method of combating tomato damping-off by dipping the seed in copper sulphate solution, marked success was obtained with spinach. Treatment consists in soaking the seed for 1 hour in a solution made by dissolving 1 to 2 oz. of $CuSO_4$ in 1 gal. of water. Considerable leeway was found permissible in the matter of concentration and of time of immersion. Increases in stand of from 20 to 75 per cent were common on the sandy-loam soils of Long Island. The average increase in yield on 8 farms was 201 crates per acre, or 65 per cent.

An inexpensive dry treatment was found just as effective as the soak. It consists in shaking the seeds for 5 minutes with cuprous oxide (Cu_2O) at the rate of from 3 to 6 oz. of the dust to 10 lb. of seed.

During the past 2 seasons over 10,000 lb. of spinach seed have been treated in Nassau County. Seedsmen, canners, and growers are now successfully treating spinach seed in advance of the planting season.

Prevalence and relations of 4 soil-borne parasites on the peach trees in the Sand Hills of the Carolinas. R. F. POOLE.

Armillaria mellea, Bacterium tumefaciens, and *Heterodera radicicola,* recognized parasites of the bole and root system of peach, along with *Polyporus curtisii,* the cause of "punky" or heart rot, are widely distributed in the Sand Hill soils. All 4 organisms are rarely observed in the same orchard, but 1 or more of the organisms occur in 90 per cent of the orchards.

Every season these organisms kill from 3 to 5 per cent of the trees, seriously affecting the economic production of the peach crop in the sand hills.

These organisms are, in most instances, indirectly responsible for the death of trees, amounting to 10 to 100 per cent in many orchards following exposures to such low temperatures as 10 and 15° F. in March and early April. Studies of the root systems of dead trees revealed diseased conditions that had prevailed some time previous to the low-temperature injury.

A complex virosis of tobacco. H. M. QUANJER.

It is well known that a "streak" of tomato can be caused by the combined action of a "true" tobacco-mosaic virus and a "healthy" potato virus. A streak, however, can also be produced in tobacco in the same way. The Amersfoorter variety of tobacco, the same as used by Adolf Mayer (1885) and Beyerinck (1896), was inoculated with the classic tobacco-mosaic virus of these authors and at the same time with the "acronecrose virus" (Phytopath. 21: 577–613. 1931) of apparently healthy Magdeburger Blaue potato. Symptoms of tobacco mosaic appeared in the tops of the plants, and in the bigger leaves the faint, nebulous, later disappearing ring spots, characteristic of the acronecrose virus, appeared. In addition, there occurred a heavy streak on the midveins of the bigger leaves and on the lower half of the stems.

The thermal death point of the classic tobacco virus is 88° C., and the time it may survive in plant extracts at 18° C. is several months; the thermal death point of the acronecrose virus is 66° C., and its longevity in plant extracts at the same temperature is 10 days.

Fungicidal efficiency of a solution of mercuric chloride and potassium iodide. W. P. RALEIGH.

Laboratory tests and preliminary field experiments indicate that the addition of potassium iodide to a solution of mercuric chloride greatly increases the fungicidal effi-

ciency of the solution when used to control Rhizoctonia on potatoes. A 1: 1,200 solution of mercuric chloride, to which 0.25 per cent potassium iodide was added, when used as a dip treatment, proved to be almost as efficient in control of Rhizoctonia as a 1: 1,000 solution of mercuric chloride used as a 1½-hour soak. It was more efficient than a 1: 500 solution of mercuric chloride used as a 3-minute soak or a 1: 500 solution of mercuric chloride, with 1 per cent commercial hydrochloric acid, used as a dip. A 2 per cent solution of potassium iodide, as a dip, did not give satisfactory control of Rhizoctonia in laboratory tests.

The rate of loss of strength of this solution with continuous use has not been determined. Injurious effects have not been noted in preliminary field tests.

A homemade colloidal copper spray. W. P. RALEIGH.

An attempt has been made to perfect a method of home manufacture of a colloidal copper spray. In preliminary tests several procedures were followed and many colloidal materials were used in an effort to get a concentrated mixture in which the copper was in a finely divided state. One of the most promising concentrates was made in the following manner: One pound of copper sulphate was dissolved in 2 qt. of water and 1 pt. of black-strap molasses was added. This mixture was stirred well and made slightly alkaline by adding about 4/10 of a pound of lye dissolved in 1 qt. of water. This concentrate was allowed to stand until it turned yellow, which usually required about a day at room temperature. In preliminary field tests this mixture, used at the rate of the equivalent of 4 lb. of copper sulphate to 50 gal. of water, gave satisfactory control of late blight of potatoes. It is especially adapted to conditions where excessive coverage of the foliage is not desired. Mosaic may be readily read in potato fields sprayed with this material, whereas it is often difficult to read it in fields sprayed with Bordeaux mixture. The spraying procedure is very simple after a quantity of the concentrate is made up.

Seasonal development of hairy root, crown gall, and wound overgrowth on apple trees in the nursery. A. J. RIKER and E. M. HILDEBRAND.

The seasonal development of hairy root, crown gall, and wound overgrowth on nursery apple trees has been studied for 3 years, in a region where hairy root was of considerable economic importance. Although weather conditions during the 3 growing seasons were quite different, the development of disease was similar. Records were kept of such factors as environmental conditions, host development, incubation periods following special treatments, successive stages in the development of artificially induced enlargements, natural occurrence of overgrowths, and insect relations. Incubation periods for the various enlargements were shorter in the summer than in the spring and fall. A relatively small portion of the natural occurrence of hairy root was traced to infection at the time of grafting. However, a large part of the natural occurrence of hairy root seemed to follow natural infection during the latter part of the first growing season and during later growing seasons. Much of the natural infection appeared correlated with such factors as warm weather, active growth of the nursery trees, short incubation periods, decreased protection of the unions by wrappers, and insect activity.

Fusarium strains in relation to wilt of China aster. REGINA S. RIKER and L. R. JONES.

Various strains of Fusaria from wilting asters, *Callistephus chinensis,* received either from Wollenweber (Europe) or Hemmi or Fukushi (Japan), have been tested for pathogenicity in comparison with Beach and Jackson strains of *F. conglutinans* v. *callistephi* with the following results.

Pathogenic: from Europe, *F. conglutinans* v. *callistephi*, *F. conglutinans* v. *majus* (2 strains), *F. oxysporum* f. *6* (4 strains), *F. lateritium* v. *fructigenum;* from Japan, unidentified (3 strains).

Nonpathogenic: from Europe, *F. oxysporum* v. *aurantiacum*, *F. redolens* (2 strains), *F. vasinfectum* v. *lutulatum*, *F. herbarum* f. *1* and f. *2*, *F. herbarum* v. *avenaceum*, *F. scirpi* v. *acuminatum*, *F. equiseti* (2 strains), *F. culmorum*, *F. solani* v. *martii;* from Japan, unidentified (2 strains).

Certain Fusaria, pathogenic on other hosts, proved nonpathogenic on aster, namely: *F. conglutinans*, *F. conglutinans* v. *betae*, *F. niveum*, *F. orthoceras* v. *pisi*, *F. tracheiphilum*, *F.* sp. from celery, *F. sporotrichioides*, *F. anguioides*, *F. arthrosporioides*.

The Wisconsin "wilt-resistant" asters proved resistant to the pathogenic Fusaria listed above, both in greenhouse and field trials, as far as tested, namely: *F. conglutinans* v. *callistephi* (3 strains), *F. conglutinans* v. *majus* (1 strain), *F. oxysporum* f. *6* (3 strains), *F. lateritium* v. *fructigenum* and 2 Japanese strains.

Control of hairy root, crown gall, and other malformations at the unions of piece-root grafted apple trees. A. J. RIKER, G. W. KEITT, E. M. HILDEBRAND, and W. M. BANFIELD.

The control of graft knots at the unions of nursery apple trees, grown from piece-root grafts, has been studied during the past 7 years in the Middle West. The results from the various control methods tried have shown considerable variations in different localities during the same year and in the same locality during different years. Different kinds of enlargements were found in various sorts and degrees of mixtures. Well-matched tongue grafts produced on the average as many trees with smooth unions as did wedge grafts. Of the antiseptics employed mercuric chloride, applied before grafting in a 1–1,000 solution to roots that carried pathogenic bacteria, appeared to have the greatest promise. Adhesive-tape wrappers for piece-root apple grafts appeared to be better than any other single control measure employed. Comparisons of the percentages of smooth trees from grafts wrapped with other commercial wrappers and those from tape wrappers, respectively, show the following average results: on trees 1 year old, 57 to 92; on trees 2 years old, 75 to 91; on trees 3 years old, 73 to 85; and on trees 4 years old, 21 to 53.

The genetics of Sphacelotheca sorghi and S. cruenta. H. A. RODENHISER.

Studies have been made of the segregates from hybrid chlamydospores formed as a result of a cross between a monosporidial line of form 1, *Sphacelotheca sorghi*, and one of a collection of *S. cruenta*. One hundred and two gametic F_1 lines segregated, as regards color of colony, into 4 general groups. Color apparently was governed by multiple factors that segregated independently of those governing sex, species, and rate and type of growth. In interspecific crosses, the factor or factors determining the *cruenta* type of sorus are dominant. Ninety-nine F_1 monosporidial lines isolated from 39 hybrid chlamydospores were paired in back crosses with one of two monosporidial lines of opposite sex of the recessive parent, *S. sorghi*, and used to inoculate Reed kafir sorghum. Segregation was observed among the back-crossed progenies for factors governing species characters as well as the color of the peridia surrounding the sori, the degree to which the plants were stunted, and the amount of vegetative tissue destroyed in the panicle.

Pathogenicity of certain hybrids of covered and loose smuts of sorghum. H. A. RODENHISER and B. F. BARNES.

Intraspecific crosses were made by pairing monosporidial lines of physiologic forms 1 and 2 and of 1 and 3 of *Sphacelotheca sorghi*. Likewise, interspecific hybrids were

made by pairing a monosporidial line of form 2 and one of form 3, respectively, with a monosporidial line of *S. cruenta*. On Reed kafir (C. I. 628), which is susceptible to all known forms of *S. sorghi* and to *S. cruenta*, the intraspecific crosses were more virulent than were inbred lines of either parental form. Interspecific hybrids were less virulent on this variety than were the inbred parental lines. In general, on the varieties of sorghum susceptible to one inbred parent and resistant to the other, both intra- and interspecific F_1 chlamydospores proved to be intermediate in their virulence. On kafir × feterita (H. C. 2423), the interspecific hybrid, form 3 of *S. sorghi* crossed with *S. cruenta*, was only slightly less virulent than either parent. Spur feterita (C. I. 623) was immune in all pathogenicity tests.

(Cooperative investigations between the Division of Cereal Crops and Diseases and Division of Dry Land Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture).

Comparative studies of field collections of Ustilago hordei and U. nuda. MABEL L. RUTTLE-NEBEL.

A summary of head and spore types obtained from the examination of 150 heads of *Ustilago hordei* and *U. nuda* collected near Geneva, N. Y.

No.	Head	Spore mass		Individual spores		Remarks
	Type	Compactness	Color	Wall	Germination	
1	Covered	Compact	Very dark chocolate brown	Smooth	Promycelia and sporidia	Typical *U. hordei*
2	Covered	Less compact	"	Echinulate	Promycelia and sporidia	Sp. ?
3	Loose	Powdery to compact	Dark chocolate brown	Smooth	Promycelia and sporidia	Sp. ?
4	Loose	Powdery to compact	Less dark chocolate brown	Faintly to distinctly echinulate	Promycelia and sporidia	*U. nigra* Tapke? Blossom inoculation gave no infection
5	Loose	Powdery	"	Echinulate	Much-branched germ tubes	Sp.? Seed had been dusted with Ceresan
6	Loose	Powdery	Olivaceous brown	Echinulate	Much-branched germ tubes	*U. nuda* ?
7	Loose	Powdery	"	Echinulate	Germ tubes not so branched as in 5 and 6	Typical *U. nuda*. Blossom inoculation produced 100 per cent infected seed

Notes on Bordeaux mixture. GEORGE E. SANDERS.

The addition of an excess of lime to Bordeaux mixture increases its value in the control of early blight of potatoes. In a modification of the Kedzie formula, it was

found that mixing the arsenite of soda solution with the copper sulphate solution and combining the majority of the arsenic with the copper before the addition of lime result in a much safer arsenical than can be obtained by adding arsenite of soda to the completed Bordeaux mixture.

Because of the lack of agitation in traction sprayers when the hydrated lime is added to the copper sulphate solution, a type of Bordeaux mixture results that is inferior to that resulting from the old method of pouring diluted solutions together. Fertilizing elements made up of synthetic urea and monopotassium phosphate added to standard Bordeaux mixture show that 1 lb. of urea and 3 lb. of monopotassium phosphate may be used with safety in 50 gal. of Bordeaux mixture. The results indicate that considerable economy in the use of fertilizers may result from this procedure.

A new necrotic virus disease of potatoes. E. S. SCHULTZ and W. P. RALEIGH.

A few necrotic potato plants of the variety British Queen were found in a field near Eureka, California, in 1929. This necrosis, called British Queen streak, is manifested by fine necrotic streaking of leaf veins and petioles and by slight streaking on stems. It differs from top necrosis or seedling streak, inasmuch as it involves at first the older leaves and causes smaller necrotic lesions than either top-necrosis or stipple streak. Moreover, no tuber necrosis has been observed.

Leaf-rubbing inoculations to Turkish tobacco within 10 days produced local, not invariably systemic, and at first light green circular areas followed by white and finally by brown spots. Similar inoculations to Globe tomato and *Datura stramonium* resulted in local and systemic infection, manifested at first by light green and finally by necrotic, circular spots. Necrosis appeared sooner at 60° to 65° F. than at 70° to 75° F. Although the symptoms of British Queen streak on tobacco and tomato resemble those figured for spotted wilt of tomato in Australia, observations indicate that these diseases may not be identical.

Resistance of potato to latent mosaic. E. S. SCHULTZ and W. P. RALEIGH.

Leaf-rubbing and tuber-graft inoculations with latent mosaic, which is generally present in masked condition in Green Mountains and other important potato varieties, have failed to infect certain potato seedlings, while similar inoculations on other seedlings have infected every plant. Under field conditions these potato seedlings have reacted like the artificially inoculated plants, viz., certain seedlings have not contracted latent mosaic, while others similarly exposed became infected. Observations on the reaction of different potato varieties and seedlings to latent mosaic indicate that some manifest this disease as severe necrosis, while others are very resistant to it.

Investigations involving isolation of different types of mosaic have shown that resistant potato seedlings can be used in isolating streak and mild, leaf-rolling and other mosaic types from latent mosaic.

Resistance of rosaceous plants to fire blight. LUTHER SHAW.

The relative fire-blight resistance of various rosaceous plants was determined by a standardized procedure, which involved artificial inoculations of the shoots with *Bacillus amylovorus* and measurements of the fire-blight lesions. Differences in resistance, ranging from slight necrosis around the points of inoculation to death of entire shoots, were found among potted plants of 31 rosaceous species, involving the following genera: Amelanchier, Aronia, Cotoneaster, Crataegus, Malus, Pyrus, and Sorbus. Among certain apple varieties studied under both greenhouse and orchard conditions, Northwestern

Greening consistently exhibited high and Yellow Transparent low resistance. McIntosh and Wealthy were intermediate.

The following environmental conditions were found to increase fire-blight resistance in potted apple plants: (1) low soil nutrients, (2) relatively low and relatively high soil temperatures, (3) relatively high atmospheric temperatures, and (4) relatively low soil moistures. These conditions also resulted in lessened shoot growth. Subjecting plants to 95–98 per cent relative humidity, at 24° C., for varying periods following inoculation greatly lowered their resistance to fire blight.

Positive correlations were found between fire-blight resistance in apple shoots and (1) a low moisture content in the shoots and (2) rapid corking off of the fire-blight lesions.

Relation of growth cycle and nutrition to perennial apple-canker infection. ELMER V. SHEAR and J. S. COOLEY.

Experimental work on perennial apple canker (*Gloeosporium perennans*) has been carried on in the experiment-station orchard at Hood River, Oregon. Hand inoculations with several strains of the organism have been made at 2- or 3-week intervals throughout the year. Infection took place in normal living twigs for a relatively short time only, namely, in the fall and early winter. Susceptibility increased very rapidly from nothing the last of September to approximately 50 per cent early in October and reached its maximum of near 100 per cent in November, where it remained until about mid-December. Mid-December infections were irregular, and, by mid-January, infection could not usually be effected on limbs of 25 or more millimeters in diameter on mature trees. However, on small limbs and on young trees immunity was later and more irregular in its development than on larger limbs. Internodes were less susceptible than nodes.

The influence of nutrition on resistance to canker was determined by inoculating trees having received different fertilizer treatments. Results indicate that trees that had received an excess of nitrate and, likewise, the devitalized trees, such as the checks and those receiving no nitrate, were more susceptible than those in good vegetative condition.

Relationship of Fusarium niveum to the formation of tyloses in melon plants. BAILEY SLEETH.

One- to 4-months-old melon plants (*Citrullus vulgaris*) infected with *Fusarium niveum* show an abundance of tyloses. The abundance of tyloses seems to be correlated with the presence, the quantity, and the proximity of the fungus. Of the infected vascular bundles of the same plant ¼ to all of the vessels may be completely filled with tyloses, while few or none occur in the uninfected bundles. Evidence indicates that the formation of tyloses either precedes or coincides with the spread of the fungus through the xylem. Few or no tyloses were found in fungus-free and apparently normal plants. The striking difference in the number of tyloses in infected and noninfected plants strongly suggests a pathological relationship between the fungus and the formation of tyloses with the subsequent plugging of the vessels. When this mechanical plugging of the vessels becomes sufficient to interfere with the normal flow of water and nutrients in the plant, stunting and wilting occur.

Association of Cercosporella foot rot with a specific ecological area. RODERICK SPRAGUE.

In the United States, the Cercosporella foot rot of wheat, *C. herpotrichoides*, occurs in certain prairie sections of Oregon, Washington, and Idaho. With some exceptions, it is confined to fine or very fine sandy-loam soils, which, in pre-farming days, were covered by a grass sod in which *Festuca* spp. predominated. It is possible

to predict the approximate range of this disease by a study of the vegetation in these prairie areas. Prominent plants in the disease-harboring sections include *F. idahoensis, Agropyron* spp., *Poa sandbergii, Koeleria cristata, Balsamorhiza deltoidea, Olsynium grandiflorum, Tellima* spp., *Fritillaria pudica, Gaillardia aristata* and various species of Lupinus and Cogswellia (Lomatium). (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and Washington agricultural experiment stations).

Distribution of physiologic forms of Puccinia graminis tritici in relation to stem-rust epidemiology in 1932. E. C. STAKMAN, LEE HINES, HARRY G. UKKELBERG, and WALLACE BUTLER.

Durum wheats were more severely rusted than bread wheats in the upper Mississippi Valley in 1932, although the opposite usually is true. This is accounted for by the distribution of physiologic forms of *Puccinia graminis tritici.* Fifteen forms were isolated from 465 collections made in northern Mexico and United States. Five forms constituted 92 per cent of all collections, as follows: form 38, 46 per cent; 49, 27 per cent; 36, 9 per cent; 19, 5 per cent; 11, 5 per cent. Forms 38 and 19 infect most hard-red-spring wheats only slightly but often infect durums heavily; the reverse is true of forms 49 and 36, while form 11 infects both groups heavily. Following are the percentages of collections in representative areas from which were isolated forms that infect durums: Mexico, 54 per cent; Texas, 67; Minnesota, 61; North Dakota, 56. Of the Mexican and Texas collections, 54 and 63 per cent, respectively, are harmless to most hard-red-spring wheats in the field. Field observations and results of spore trapping support evidence from the physiologic-form survey that rust was blown northward from Mexico and Texas, although certain forms, notably form 36, apparently originated principally on northern barberries. (Cooperative investigations between the Divisions of Barberry Eradication and Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station).

Some experiments with a yellow mosaic of tomato. W. G. STOVER and M. T. VERMILLION.

The chlorotic areas in leaves of diseased plants are irregular in shape and much lighter in color than in ordinary mosaic. Thus far, successful inoculations have been made in tomato, tobacco, pepper, and *Solanum nigrum.* Susceptibility of *S. dulcamara* is in doubt. Inoculations in China aster, burdock, catnip, cucumber, and curly dock gave negative results. The disease is much more injurious to pepper than ordinary mosaic. The leaves and blossom buds drop off and many of the plants are killed. The virus of yellow mosaic was inactivated at about 83° C.; that of ordinary tomato mosaic at about 90° C. in these experiments. "Streak" is induced when healthy tomato plants are double-inoculated from tomato plants with yellow mosaic and from potato plants.

Evidence regarding the shape of tobacco-mosaic-virus particles. WILLIAM N. TAKAHASHI and T. E. RAWLINS.

If a liquid containing small rod-shape particles flows from a small tube the entire stream should exhibit form double refraction; if the liquid is sucked from a beaker into the tube the flowing doubly refractive liquid in the beaker should exhibit a dark cross. A liquid containing small discs or leaf-shape particles should show form double refraction only near the margins of the stream and no double refraction or dark cross when sucked from a beaker into a tube.

Sols, the particle shape of which had been determined by Freundlich and associates by the application of the Tyndall phenomenon, were expelled from and drawn into a

small tube as described above; all sols tested exhibited the expected double refraction phenomena, thus confirming the above hypotheses. Tobacco-mosaic virus exhibited the double refraction characteristic of sols containing rod-shape particles; juice from healthy plants exhibited no double refraction.

Preliminary studies have indicated that the particles in the infective tobacco juice that cause the double refraction have size and precipitation properties characteristic of the virus. The available evidence, therefore, indicates that tobacco-mosaic virus is composed of rod-shape particles.

Comparative studies of two loose smuts of barley. V. F. TAPKE.

Stilbum cinnabarinum, the cause of a new fig disease in Louisiana. E. C. TIMS.

In May, 1932, diseased fig branches were received from Ascension Parish, Louisiana. These twigs were covered with small reddish pink fungous heads borne on short synnemeta. The heads consisted of masses of small, hyaline, unicellular conidia, from which pure cultures were easily obtained. Inoculations made in young fig branches through small wounds developed the typical fungous heads 20 days later. The fungus spread rapidly through the woody tissues of the twig and into the pithy center. A number of the inoculated twigs had been killed back to the 2-year-old wood after 30 days. The fungus was reisolated, and numerous inoculations established its parasitism. The fungus causing this twig blight of fig has been identified as *Stilbum cinnabarinum*. Observations made during the summer of 1932 show that the disease is wide-spread over southwestern Louisiana, on the Celeste fig, but apparently causes only minor damage under most conditions.

Leptosphaeria salvinii, the ascigerous stage of Sclerotium oryzae. E. C. TULLIS.

In the summer of 1931 an ascomycete belonging to the genus Leptosphaeria was found on rice plants in the greenhouse at Fayetteville, Arkansas. The fungus proved to be *Leptosphaeria salvinii*. The same fungus was found on 16 different varieties of rice at the Rice Branch Experiment Station near Stuttgart, Arkansas. Cultures from ascospores in the winter and spring of 1931–32 produced sclerotia typical of *Sclerotium oryzae* and conidia typical of *Helminthosporium sigmoideum*. Rice plants were inoculated with sclerotia from colonies originating from ascospores. Perithecia, conidia, and sclerotia were produced on these plants. Thus, the genetic connection between these three stages has been established by cultural and inoculation experiments. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas, Louisiana, and Texas agricultural experiment stations).

Further purification of the virus of tobacco mosaic. CARL G. VINSON.

Progressively increasing the amount of a safranin solution added to a given amount of juice from diseased plants results in a progressive decrease in concentration of virus remaining in the supernatant liquid.

The virus may be reprecipitated with safranin without appreciable loss of virus being demonstrable.

When two volumes of acetone are added in the cold to one volume of the virus fraction obtained on decomposing the safranin precipitate, a precipitate forms; but considerable virus seems to remain in the supernatant liquid.

Comparative studies of peas resistant and susceptible to Fusarium wilt. J. C. WALKER.

Resistant and susceptible strains of Alaska were used. Plants were grown for various intervals at about 15° C. in Tottingham's solution, to which was added, after plant removal, 1 per cent dextrose. The pea-wilt fungus, *Fusarium orthoceras* var. *pisi,* was grown on aliquot portions of the autoclaved solutions. There was observed very consistently a heavier growth in the solution in which the susceptible roots had grown. In autoclaved extracts from fresh susceptible roots there was a slightly heavier growth than in those from resistant roots. In extracts from air-dried roots a similar slight difference was obtained. In extracts from roots dried at 85° C. there was a markedly heavier growth in those from susceptible roots.

Relation of soil fertility to incidence of Aphanomyces root rot of pea. J. C. WALKER.

Colby silt loam is one of the most favorable Wisconsin soils for the development of this disease. Eight canning varieties were tested in the greenhouse on this type of soil from a heavily infested field. All were severely damaged by root rot. When a 500-lb. application of 4–16–4 fertilizer was added to a portion of this soil there was a marked reduction in the severity of the disease. In the Alaska variety 63 to 100 per cent of the plants were severely infected in the nonfertilized soil, while 3 to 20 per cent were so affected in the fertilized soil. Surprise, Bruce, Admiral, Ashford, Perfection, Prince of Wales, and Wisconsin Early Sweet reacted in a similar manner.

Nectria canker of basswood. D. S. WELCH.

Cankers have been found seriously affecting the basswood, *Tilia americana,* in native stands in central New York. On 2 half-acre plots selected at random there were 258 trees, 117 of which bore cankers of the type commonly associated with Nectria infections on other trees. The lesions are sunken in the center and a number of concentric callus rolls marks the age of the infection. A species of Nectria has been repeatedly isolated from infected tissue at the margin of the cankers and from conidia and perithecia occurring on affected tissue. Basswood trees of various ages have been inoculated with positive results in 54 out of 64 trials. All 7 checks remained healthy. The same organism has been reisolated several times from such infections 18 to 24 months after the date of inoculation. Stromata bearing conidia and perithecia are produced sparingly at the margins of cankers on living trees. On infected trunks that have been cut and left in the woods the fungus develops in great abundance. On such trees perithecia containing viable spores may be found every month throughout the year. Similar diseases, apparently hitherto unreported, have been found on *Acer rubrum, A. spicatum, Fraxinus nigra, Populus grandidentata, Prunus serotina,* and *Rhus typhina.*

Relation of cranberry varieties to the spread of false blossom. R. B. WILCOX.

The severity and rate of spread of false blossom in a cranberry-growing region are related somewhat to the proportion of the total acreage devoted to resistant varieties. In New Jersey there have been, during the past decade, wide differences between varieties as to losses suffered. A quick and convenient method has been developed that seems to test accurately the resistance of a variety to the spread of false blossom.

Bacterial canker of Prunus spp. in California. E. E. WILSON.

A disease in California that has been regarded as the "bacterial gummosis" described in Oregon by Griffin and Barss may be produced by two types of bacteria that differ in chromogenesis and possibly in temperature requirements but are similar in

most other characters. In the pathogenicity and cultural tests so far attempted one of these organisms agrees in most characters with those described for *Pseudomonas cerasi*. The other appears to be a strain of *Ps. cerasi*. In comparative tests, this strain and *Ps. prunicola*, the latter recently described by Wormald, from England, differed only in size.

The cankers of the disease are quiescent during most of the summer but begin to extend at about the time of leaf fall in the autumn. This activity continues throughout the winter and early spring but wanes soon after the trees come into leaf. While temperature is found to influence the rate of canker extension, it apparently is not the only cause of this seasonal variation in activity. Evidence obtained by inoculating plums throughout the autumn, winter, and spring suggests that the host itself may influence canker extension.

Development and control of olive knot. E. E. WILSON.

Olive knot has become serious in the northern part of the Sacramento Valley within the last 4 years. It is found on trunks, limbs, smaller branches, leaves, and occasionally on fruits. It occurs most abundantly on the smaller branches.

In certain orchards 95 per cent of the knots develop at leaf or blossom scars. The time of leaf fall and the variations in the amount of leaf fall from growth of different age and from trees of different vigor have been studied with respect to their effect on leaf-scar infection.

Through inoculations it was determined that, although infection may take place in December and January, the knots may not appear until March or April. On the other hand, inoculations made in March and April developed knots within a few weeks. The same situation was reflected in the development of knots resulting from natural infection, inasmuch as nearly all knots appeared in the spring and early summer.

Control has been attempted through application of the following materials as sprays: lime sulphur, sodium fluosilicate, zinc sulphate lime, commercial Bordeaux with an oil emulsion, and homemade Bordeaux. The latter material gave promise when it was used in 3 applications. The other materials gave varying degrees of control but were, on the whole, unsatisfactory.

Influence of spray materials on transpiration. J. D. WILSON and H. A. RUNNELS.

Bordeaux mixture checked growth, decreased yield, and increased transpiration and blossom-end rot of tomatoes, whereas none of these conditions obtained in an oil spray. Experimental data collected to date indicate that the following relations probably exist: (1) Transpiration is accelerated by Bordeaux mixture more during the night than the day period. (2) Certain other copper sprays do not cause increases in transpiration comparable with Bordeaux mixture. (3) If the quantity of copper in a given volume of Bordeaux mixture is held constant, each additional increment of lime increases the influence of the mixture on the transpiration rate until the quantity of lime becomes about 1½ times as great as that of the copper. (4) Bordeaux mixtures made up with high magnesium and with high calcium limes are equally effective in increasing transpiration. (5) A mixture of hydrated lime and water is not so effective as a Bordeaux mixture containing an equivalent amount of lime. (6) Mixtures in which the particles remain well in suspension are more effective than those in which settling occurs quickly. (7) Certain other sulphates in combination with hydrated lime are as effective as copper sulphate.

Use of formaldehyde dust with vegetable seedlings. J. D. WILSON and P. E. TILFORD.

Formaldehyde dust has been found to improve the stand of seedlings in those soils in which damping-off occurs if no precautions are taken to prevent it. When the 6 per cent dust is mixed with the soil at the rate of 8 oz. per bu., damping-off is controlled and formaldehyde injury to the seeds themselves is rare. If the dust is placed in the seed row at the rate of 1 gm. per linear ft., the stand of certain vegetables, especially spinach and beets, is improved, whereas such plants as the crucifers are injured by the formaldehyde.

Formaldehyde is least toxic when adsorbed on inert carriers of low weight per unit of volume, such as infusorial earth and finely divided charcoal. Injury to seeds is greatest in heavy soils and those low in organic matter. Also, injury is greatest at medium soil-moisture content. It has been found that in most cases seedlings cannot be transplanted into treated soil until at least 72 hours after treatment. Formaldehyde escapes more quickly from dry and warm soils than from cold and wet ones. If transplanting is done immediately after treatment, less injury occurs in soils of high absorptive capacities, such as muck.

Nature of rust resistance in beans. S. A. WINGARD.

Bean varieties differ markedly in their response to rust and may be grouped roughly into 3 classes on the basis of their reaction to infection. (1) Varieties that are immune, showing no evidence of infection; (2) those that show severe flecking as a result of infection; and (3) those on which numerous fertile sori are produced. The varieties in the first group show true resistance; those in the second and third groups are susceptible. The flecking varieties (Group 2) are hypersensitive to rust infection, and, as a result, the rust fungus immediately kills the invaded tissues upon entering the leaf and thereby sets up a condition detrimental to the further development of the fungus. In the third group the rust fungus and host tissues seem more compatible and are thus able to endure each other. In this group there is evidence that the rust fungus acts even as a preservative for the invaded tissues of the host. This is shown by the fact that the region immediately surrounding the sorus is the last part of the leaf to lose its chlorophyll and die.

Intracellular bodies in ring spot. MARK W. WOODS.

Intracellular bodies, similar to those in tobacco mosaic, have been found in ring spot. They were found in primary and systemic lesions in *Nicotiana tabacum* var. Turkish and in primary lesions in *N. tabacum* var. Havana Seed-Leaf, *N. rustica*, *N. glutinosa*, and Petunia. Best results were obtained after fixation in formol-acetic alcohol and staining with Fleming's triple stain. The bodies appeared typically granular in structure, vacuolate, and with or without membrane-like surfaces. In Nicotiana they sometimes contained minute red cuboidal inclusions. Similar cuboidal bodies were also found in the cytoplasm and nuclei of diseased and healthy cells. They appear to be similar to cuboidal structures observed by certain workers in mosaic-diseased and healthy tobacco plants. Intracellular bodies were found in cells of the mesophyll, epidermis, and trichomes. The development of intracellular bodies seemed to be directly related to the formation of visibly lesioned areas in the leaf. Bodies were never found in normal-appearing regions of diseased or healthy leaves. Intracellular bodies as large as the nucleus were observed in lesions 5 days after inoculation, but bodies were more commonly found in older lesions. Tests with Turkish tobacco and *N. glutinosa* apparently demonstrated that no virus other than that of ring spot was present in plants in which intracellular bodies were found.

Sclerotinia stem canker of hollyhock. PAUL A. YOUNG.

A fungus with the characters of *Sclerotinia libertiana* was isolated in 1931 from stem cankers in hollyhock, *Althaea rosea,* in Bozeman, Montana. In 1932 it was inoculated into wounds in hollyhock and White Beauty sunflower, *Helianthus annuus,* stems growing in a field plot. It caused many cankers within 13 days and killed the infected stems within 60 days. Seventy-one of the 84 hollyhock inoculations and 21 of the 27 sunflower inoculations caused cankers. *Sclerotinia libertiana* was reisolated from the hollyhock and sunflower cankers. Only the sunflower cankers spread into the roots during the summer. The sunflower cankers were like those described in Montana Bulletin 208. The hollyhock cankers from natural and artificial inoculations were white, 5 to 45 cm. long, and usually encircled the stems. Many cankers had black margins and bore 1 to 4 brown, concentric arc lines each about 1 by 10 mm. The arcs often marked epidermal wrinkles. Black streaks occurred in the epidermis and wood near some cankers. Black sclerotia occurred in cavities formed in the stem pith. Sclerotia occurred on cankers in hollyhock stems that had been under snow.

Soil infestation by chlamydospores of Tilletia levis in Montana. PAUL A. YOUNG.

Seed wheat, surface sterilized with formaldehyde, was planted by scattering from envelopes into 3 m. rows at about 10-day intervals on 4 dates in September and 3 dates in October, 1931. The rows were in 4 fallowed plots in a region 4 km. square near Menard. The rows were within 10 to 50 m. from one of six 50- to 300-acre-wheat fields, combine-harvested in August, 1931, in which 12 to 50 per cent of the heads were bunted by Tilletia. Wheat planted before October 6 emerged within 15 to 30 days. Soil infestation by Tilletia caused the following average percentages of bunted heads in the following varieties of wheat planted in September, 1931: Turkey, 11.9; Oro, 0.8; Regal, 0.9; Kharkof, 2.7; Sherman, 0.6; Ridit, 0.2; Hybrid 128, 12.5; Albit, 0.2; Hussar, 0.2; Kanred, 12.1; Montana 36, 20.0; and Jones Fife, 21.9. The Tilletia (*T. levis*) concerned probably was physiologic form 3 of Oregon Bulletin 281. Smutted Montana 36, planted as checks, averaged 65 per cent bunt. Soil infestation by Tilletia decreased rapidly in October, becoming 0.

(Cooperative investigation between the Botany Department of the Montana Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture).

Transmissibility of certain legume-mosaic viruses to bean. W. J. ZAUMEYER.

Cross-inoculation experiments have shown that the viruses causing mosaic diseases of several legumes are capable of being transmitted to beans. The viruses causing mosaics of white sweet clover, *Melilotus alba,* yellow sweet clover, *M. officinalis,* white clover, *Trifolium repens,* and alsike clover, *T. hybridum,* gave positive results when inoculated to beans. A mosaic virus from red clover, *T. pratense,* gave negative results.

The virus of the mosaic of white sweet clover produced symptoms on beans slightly different from those of the common bean mosaic. It is possible that some of the secondary spread of bean mosaic in the field is the result of aphid transmission from the white-sweet-clover-mosaic virus.

The virus of the mosaics of pea, *Pisum sativum,* and of sweet pea, *Lathyrus odoratus,* gave positive results when inoculated into beans, either by means of expressed juices from diseased plants or by the pea aphis, *Illinoia pisi.*

Transmission of bean-mosaic virus by insects. W. J. ZAUMEYER.

Insect-population studies in bean fields in Colorado, Idaho, and Virginia, conducted for the past few years by the Bureau of Plant Industry and the Bureau of Entomology of the United States Department of Agriculture, have shown many species of insects present that, with the exception of aphids, proved incapable of transmitting bean mosaic.

Various species of aphids whose number depends on the season and the locality are always present on beans in the field. The greatest population occurs at the early part of the growing season.

Under controlled greenhouse conditions, the virus has been transmitted by feeding virus-free aphids on mosaic-infected beans for 24 to 30 hours and then transferring the insects to healthy plants. In addition to the potato aphid, *Illinoia solanifolii*, the bean aphid, *Aphis rumicis*, and the peach aphid, *Myzus persicae*, shown by other investigators to be vectors, the following aphids will also transmit the virus: the pea aphid, *I. pisi*, the cucumber aphid, *A. gossypii*, the cabbage aphid, *Brevicoryne brassicae*, the Chenopodium aphid, *Hyalopterus atriplicis*, and an aphid, *Macrosiphum ambrosia*, common on wild Compositae. All these species except *M. ambrosia* have been found on beans in the field. (Aphid determinations made by P. W. Mason, Bureau of Entomology.)

STUDIES ON CERTAIN ENVIRONMENTAL RELATIONS OF THE ROOT-KNOT NEMATODE, HETERODERA RADICICOLA[1]

G. H. Godfrey and Helene Morita Hoshino

(Accepted for publication April 4, 1932)

Information on environmental relations of a plant-parasitic organism is fundamental to control. Upon attacking the problem of nematode control in connection with pineapple culture in Hawaii, it was evident that specific information was lacking concerning the relations of environment to survival of eggs and larvae of the root-knot nematode, *Heterodora radicicola* (Greef) Müller. This paper contains the results of specific studies on this problem, a brief preliminary report of which has already been made (10). The portion dealing with high-temperature relations, being a unit by itself, is reported in a separate paper (13).

The specific studies reported in this paper were stimulated by indications in the literature and from various observations and exploratory experimental work that the root-knot nematode is very highly susceptible to injury by adverse environmental conditions. In a preliminary experiment conducted at Washington, D. C., some years ago, this was strikingly shown. The soil surrounding the roots of a badly root-knotted begonia plant was divided into 3 equal portions, which were treated as follows: (1) exposed in a layer about 1 in. deep to July sun for 6 hours (10:00 a. m. to 4:00 p. m.); (2) same treatment, then remoistened, and the exposure repeated on a 2nd day; (3) held in the laboratory unexposed and continuously moist. All 3 lots were then set to small tomato plants previously grown in sterilized soil. In about 3 weeks, examination of these plants revealed that plants *1* and *2* were entirely free from infection, whereas plant *3* was heavily knotted. Definite complete killing of the nematodes in the soil, in all stages, had resulted from even the few hours' exposure of but one day. Killing was due to one or more of the three factors, heat, drying, and light. This result and the results of other exploratory experiments and literature reports indicated the desirability of determining the extent to which each of these factors, temperature, drying, and light, was capable of contributing to the mortality of nematodes in controlled life-history stages. The present paper has to do with humidity relations and with the effects of light.

Most of the contributions to the literature on this subject are very general in nature. Specific papers are cited in the introductory paragraph to each phase of the subject considered.

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CULTURE METHODS

Eggs and larvae were obtained readily, as desired, by the methods described in another paper (9).

Death of the larvae was manifested by lack of movement, and by failure to recover motility within a week, during which period conditions were favorable for life. The greatest certainty of such favorable conditions was obtained in shallow, well-aerated drops of tap water exposed to the air, though larvae in beakers of water in the ice box at about 10° C. remained alive and active for 2 weeks or longer. Drowning is a possibility, however, particularly in water from which air has been exhausted. Dead larvae lie normally in a very typical extended or slightly bowed position (Fig. 1).

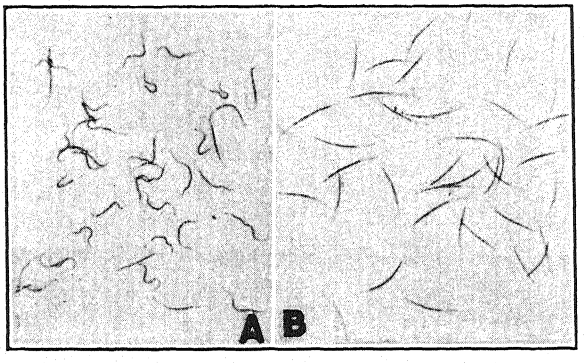


FIG. 1. *Heterodera radicicola* larvae. A. In the attitude of normal activity and B. In the attitude of death.

When all the larvae of a treatment are in this condition and do not recover movement within a few days, it is reasonably certain they are dead. In some of the tests further trials of doubtful material were made by using it as inoculum on the roots of highly susceptible cowpea plants growing in sterilized soil. If no knots developed, it was considered that there were no living larvae in the inoculating material. As the readings by observation always checked with inoculation readings, the latter were eventually eliminated.

Similar tests with eggs demanded that they hatch under favorable conditions within 30 days in order to be considered alive. Extensive tests were made to determine most favorable conditions for this viability test. Byars (3) placed eggs on plates of agar and observed presence or absence of larvae, with no particular count of percentage of hatch. McClintock (14) conducted tests on the effects of various chemicals on eggs by transferring treated egg masses to test-tubes containing synthetic-nutrient agar. After trying similar media we discarded agar in favor of the simpler

TABLE 1.—*Percentage of untreated eggs hatched at room temperature (24–28° C.)*

Lot No.	No. of egg masses	Total No. of eggs	Av. eggs per egg mass	Percentage of total eggs hatched at successive time intervals (days)							Percentage total hatch
				2	4	8	12	16	20	24	30	
1	6	1,738	290	5.29	10.24	28.94	45.05	7.07	0.11	0	0	96.3
2	6	1,410	235	7.80	9.78	33.26	32.90	12.12	0.56	0.07	0	96.5
3	12	6,254	521	14.16	17.39	24.83	23.88	16.45	0.67	0.46	0	97.7
4	12	5,228	436	25.24	27.90	34.18	9.04	2.37	0.01	0	0	98.7
5	12	4,704	392	28.23	20.79	21.68	28.08	0.14	0.02	0	0	98.9
6	12	3,719	310	36.19	30.43	22.64	3.14	0.16	0.10	0	0	95.5
7	10	4,856	486	30.91	34.67	23.02	9.08	1.07	0.24	0	0	99.0
8	4	2,977	744	10.21	10.71	12.59	46.38	17.03	2.21	0	0	99.1
9	10	4,085	409	2.76	7.90	16.59	41.90	26.56	1.66	0	0	97.4
Averages			416	20.02	21.16	23.86	23.24	8.88	0.58	0.09	0	97.8

method of drops of water on slides held at room temperature. It was found that hatched larvae migrated readily out of the egg mass and moved freely in the bottom of the drop. By transferring the egg mass to a new drop daily it was possible very readily to count the larvae and thus determine the daily hatch. A daily change of water was found advisable, as these nematodes are quickly killed by pollution.

Time required for hatching under average conditions and percentage of total number of eggs hatched in 1 series of tests are reported in table 1. Obviously this table applies only to this particular series. Rate of hatching depends very much upon the temperature. Under somewhat warmer conditions, the time required for complete hatching may be shorter, other conditions being the same. No attempt was made to control the age of egg masses. It is obvious from the results that, with some, the embryonic stages of cell division and multiplication were already well advanced.

Incubation conditions were maintained for 30 days, at the end of which period no further hatching had occurred for 4 to 6 consecutive days. In every lot at least 96 per cent of the eggs hatched. In several plates a 100 per cent hatch was obtained.

In addition to being a reliable method for testing viability of eggs, this preliminary work gave information on the egg-producing capacity of the root-knot nematode. In our test, as shown by table 1, the majority of the eggs had hatched by the 8th day of incubation; nearly all were hatched by the 16th day, which is somewhat faster than the time reported by Frandsen (5), 20 to 28 days. Other studies revealed individual instances of very high numbers of eggs within an egg mass, counts having been made of 1,052, 1,154, 1,277, and 1,308 in individual egg masses. The average number in this lot of 84 egg masses was 416 eggs per egg mass. Other figures reported have been 300 to 500 by Frandsen (5) and Childs (4) and 100 to 200 by Atkinson (2).

THE EFFECT ON THE VIABILITY OF EGGS AND ON THE LIFE OF THE LARVAE WHEN EXPOSED TO DIFFERENT PERCENTAGES OF RELATIVE HUMIDITY

Stone and Smith (19) stated that neither larvae nor eggs can stand desiccation, but no actual data on its effect were presented. Frandsen (5) reported that nematodes are resistant to cold but lack resistance to drying. Eggs and larvae placed on a glass slide and allowed to dry for a few minutes failed to show any signs of further development and, if infected roots and tubers were allowed to dry thoroughly, all the eggs and larvae in them were killed. Spaulding and Gravatt (17) tested the influence of physical factors, particularly drying, upon the viability of the sporidia of the white-pine-rust fungus, *Cronartium ribicola* Fischer, and reported that the viabil-

ity was decreased merely by bringing them to air-dried condition. They used desiccators, in which the relative humidities were controlled by using different concentrations of sulphuric acid, to study the effect of prolonged drying. They pointed out that the injury to the sporidia from exposure increased with the decrease in the relative humidity.

Methods. To determine the effects of drying alone on nematodes, it was necessary to maintain noninjurious light and temperature conditions. All tests were conducted in the laboratory free from direct sunlight, hence the light factor was eliminated. Room temperature (about 20 to 28° C.) shows no injurious effect upon the life of the larvae or on egg viability.

In order to control the relative humidity, technique similar to that employed by Spaulding and Gravatt (17) was followed. Scheibler desiccators of approximately 5,000 cc. total capacity were used. Sulphuric acid of 1.84 specific gravity was used in making up the solutions that would produce the following relative humidities: 50, 60, 70, 80, and 90 per cent. Stevens (18), the Handbook of Chemistry and Physics (12), and the Smithsonian Tables (16) were consulted in preparing these solutions. Density was ascertained with hydrometers. Four hundred cubic centimeters of each solution were put in the base of each desiccator. This amount of acid was used because it presented a maximum surface area for the most rapid action on the air, and with such a volume the density of the acid changes very little by the water taken up from the air of the chamber. As soon as the acid solution was put in the desiccator, the cover was sealed with vaseline and the chamber placed in a dark room where the temperature was fairly uniform. The acid was poured into the chambers 24 hours before the beginning of a test, so that the relative humidity of the air could reach equilibrium. A 100 per cent moisture condition was obtained by pouring 400 cc. of distilled water, instead of acid, into the desiccator. In making the test with larvae, 100 freshly hatched active larvae were first placed in a small drop of water on each slide and left in the laboratory for preliminary drying. As soon as the slide looked dry to the unaided eye, it was examined for water under a microscope. If no free water was detected and if all the nematodes were still moving about, the slide was immediately used for exposure. Several control slides of larvae that were thus dried in a preliminary way were put under favorable moisture conditions by adding a drop of water to each, and it was found that not a single nematode had been killed. Egg masses previously taken from roots and placed in water were dried on filter paper and placed on slides ready for exposure. For studies on free eggs, egg masses were dissected under the microscope and the freed eggs pipetted into drops of water on a slide, 100 to a slide. The slide bearing the eggs or larvae was

TABLE 2.—*Effect of exposure to different percentages of relative humidity on the life of the larvae and on egg viability*

Relative humidity percentage	Larvae		Eggs in masses		Eggs free	
	Time of exposure	Percentage killed	Time of exposure	Percentage killed	Time of exposure	Percentage killed
50	30 sec.	17	15 min.	12.02	15 min.	20.9
	1 min.	34	30 "	21.65	30 "	49.7
	3 "	94	1 hr.	98.4	1 hr.	99.2
	4 "	100	1½ "	94.2	1½ "	100.0
			2 "	99.0	2 "	100.0
			2½ "	100.0		
			3 "	100.0		
60	30 sec.	5	30 min.	2.84	15 min.	15.4
	1 min.	30	1 hr.	95.4	30 "	46.7
	3 "	77	1½ "	97.9	45 "	86.9
	5 "	100	2 "	98.8	1 hr.	96.4
			2½ "	99.8	1½ "	98.7
			3 "	100.0	2 "	99.3
					2½ "	100.0
					3 "	100.0
70	30 sec.	3	30 min.	1.4	30 min.	25.3
	1 min.	9	1 hr.	21.1	1 hr.	50.1
	3 "	26	1½ "	25.6	1½ "	51.4
	5 "	30	2 "	88.0	2 "	94.1
	7 "	56	2½ "	88.6	3 "	100.0
	8 "	86	3 "	99.8	4 "	100.0
	10 "	100.0	4 "	100.0		
	12 "	100.0				

TABLE 2—(*Continued*)

Relative humidity percentage	Larvae		Eggs in masses		Eggs free	
	Time of exposure	Percentage killed	Time of exposure	Percentage killed	Time of exposure	Percentage killed
80	30 sec.	2	30 min.	0.1	30 min.	7.5
	1 min.	3	1 hr.	1.5	1 hr.	44.1
	5 "	13	1½ "	16.1	1½ "	49.3
	8 "	60	2 "	73.6	2 "	80.1
	10 "	90	3 "	92.0	3 "	98.4
	12 "	92	4 "	92.8	4 "	100.0
	15 "	100	5 "	100.0	5 "	100.0
	20 "	100	6 "	100.0		
90	30 sec.	0	30 min.	0.2	30 min.	2.8
	1 min.	0	1 hr.	2.0	1 hr.	12.4
	5 "	2	1½ "	1.6	1½ "	43.1
	10 "	9	2 "	2.4	2 "	45.6
	12 "	12	3 "	6.2	3 "	49.2
	15 "	13	4 "	20.0	4 "	87.0
	20 "	28	5 "	21.4	5 "	99.8
	30 "	100	6 "	30.0	6 "	100.0
			7 "	98.0	7 "	100.0
			8 "	98.6		
			9 "	100.0		
100	24 hr.	0	6 hr.	0.2	8 hr.	0.9
			7 "	0	24 "	1.4
			10 "	0.5		
			24 "	1.3		

placed on the perforated porcelain plate in the container. It was inserted or taken out of the desiccator in less than 2 seconds.

After the completion of a series of tests, the density of the solution was again determined with a hydrometer. There was no observable variation in the desiccators during the experiment. Each treatment was replicated 3 times. Unexposed slides of larvae and eggs were kept in the laboratory in Petri dishes lined with moist filter paper to serve as controls.

Results and Discussion. The effects of exposure to different relative humidities on the life of the larvae and on egg viability are presented in table 2. In bold-face type in this same table can be seen, under each humidity, the shortest period at which all the nematodes were killed.

Table 2 shows that there was an increase in the number of larvae killed and in the injury to egg viability as the percentage of relative humidity decreased. Also, increase in length of time of exposure increased the in-

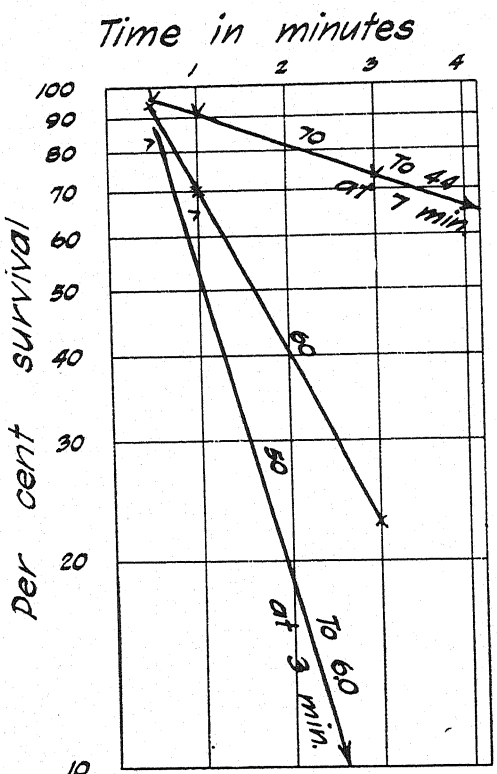


FIG. 2. Semilog graph showing percentage of survival of *Heterodera radicicola* larvae at 50, 60, and 70 per cent relative humidity. Note the relatively straight-line relationship between time and percentage of survival. Note also that at the lower humidities the rate of killing is more rapid than at the higher.

jurious effect. Relative humidity of 100 per cent completely failed to kill the larvae or injure the viability of the eggs. The data for egg masses at this humidity show a consistently high percentage of hatching. With lower humidities a longer time was required to kill the eggs than was required for the larvae; likewise a longer time was required to kill in egg masses than to kill free eggs.

The striking fact is evident from table 2 that even under relatively high humidity conditions (90 per cent) only a comparatively short period of time is required to kill even the more resistant stage (egg stage) of the nematode. Under average summer *air* conditions in Hawaii, approximately 70 per cent relative humidity, hatched larvae are killed in only 10 minutes, eggs in egg masses in 4 hours, and free eggs in 3 hours.

One phase of the results of table 2 is presented in another form in figures 2 and 3, which are semilog graphs showing the relatively straight-line relationship between time and percentage survival in larvae and in

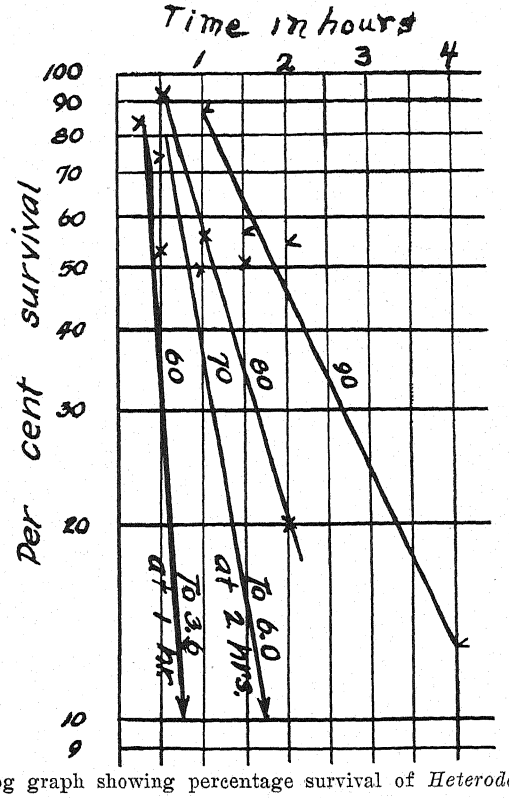


FIG. 3. Semilog graph showing percentage survival of *Heterodera radicicola* free eggs at different relative humidities for different time intervals. The story here is much the same as that shown for larvae, except that the rate of killing is much slower.

free eggs, respectively. The results with eggs in egg masses were too irregular to show such straight-line relationship, which is readily accounted for by the irregularity of the protective material of the egg mass. The degrees of slope shown by the lines in each graph as a unit indicate relative rates of killing with the different degrees of humidity. The more rapid killing at the lower humidities is readily seen. Figures 2 and 3 are not to be compared in this respect, since in the one time is plotted in minutes whereas in the other it is in hours.

THE EFFECT ON EGG VIABILITY AND ON LARVAE WHEN EXPOSED TO COMPLETE DRYNESS FOR DIFFERENT PERIODS

Methods. In performing this test, favorable light and temperature conditions were maintained. The experiments were conducted in a dark room at room temperature. Preliminary steps were identical with those followed in the tests of different degrees of relative humidity; the organisms were allowed to stand under laboratory conditions until visible free water had evaporated before they were placed in the (practically) completely dry environment, which was maintained in a desiccator by means of concentrated sulphuric acid. The desiccator was sealed with vaseline and tests conducted in the same manner as in the previous experiment. Dehydration of the organisms began, of course, immediately after the vessels were sealed.

Experimental. The larvae were exposed to complete drying for the following periods: 10 to 45 seconds and 1 to 5 minutes. The egg masses were tested for from 5 to 30 minutes and 1 to 3 hours. Table 3 indicates that exposure to complete dryness for even the brief period of 10 seconds had some injurious effect upon the life of the larvae. In 3 minutes, every

TABLE 3.—*Effect of complete drying for different periods on Heterodera radicicola*

Larvae		Eggs in masses		Eggs free	
Time of exposure	Percentage killed	Time of exposure	Percentage killed	Time of exposure	Percentage killed
10 sec.	4	5 min.	6.0	10 min.	24.2
15 "	15	10 "	9.0	15 "	50.1
30 "	25	15 "	12.4	30 "	99.6
45 "	36	20 "	14.8	45 "	99.8
1 min.	49	30 "	98.4	1 hr.	100.0
2 "	83	1 hr.	98.7	1 " 30 min.	100.0
3 "	100	1 " 30 min.	98.1		
4 "	100	2 "	100.0		
5 "	100	2 " 30 min.	100.0		

larva was killed. The eggs were not killed until after a much longer period of exposure. After 30 minutes, nearly all (98.44 per cent.) were killed, but it took an exposure of 2 hours to kill every 1 in all egg masses. The irregularity in degree of protection offered by the gelatinous matrices in different oothecae is manifest here. Free eggs were killed in 1 hour.

EFFECT ON EGG VIABILITY AND LIFE OF LARVAE WHEN EXPOSED TO SUNLIGHT AND TO LIGHT FROM A MERCURY VAPOR LAMP

The literature on effects of ultra-violet radiation on various organisms is too great to mention, except very superficially, in this paper. Much has been done on its lethal effects on various pathogenic bacteria. Spaulding and Gravatt (17) report that solar radiation, with the heat and drying factors controlled, quickly kills nearly all the exposed sporidia of *Cronartium ribicola*. Fulton and Coblentz (8) did some careful work with ultra-violet on fungi of various kinds. Sheard and Hardenbergh (15), studying the effects of ultra-violet on *Demodex folliculorum* of dogs, report that irradiation by the mercury vapor lamp at 25° C. at a distance of 50 cm. is fatal in 15 to 30 minutes. No reports of investigations have been found by the writers on the eggs or larvae of *Heterodera radicicola*. Freer (6) reports killing of nematodes, without referring to the kind, after 2 or 3 minutes' exposure to the sun. Watson (20) reports that hot sunlight is very injurious to soil organisms in general.

Methods. The technique used in the study of effects of ultra-violet on *Heterodera radicicola* was suggested by that employed by Lois Godfrey (11) in her studies on the measurement of ultra-violet in Hawaii sunlight. Some of the apparatus used in our studies was supplied by her. During the course of any specific exposure, favorable temperature and moisture conditions were maintained. Slides, each containing a fresh egg mass or 100 active larvae in a drop of water, were placed in a dish lined with moist filter paper and covered with a quartz glass top that is penetrable by ultra-violet. (It is well known that ordinary laboratory glass absorbs all except the longest wave lengths of the ultra-violet of the spectrum).

Such dishes were exposed to the sun usually on bright, clear days for various periods of time. Similar slides bearing larvae and egg masses were exposed to ultra-violet radiation for various periods of time. The source of radiation employed was a Cooper-Hewitt, air-cooled, quartz mercury vapor lamp operated at 110 volts, exposure being at a distance of 50 cm.

A dark glass known technically as "Corex red purple" (Corning Glass Company No. G. 986A), known to exclude practically all the solar rays except the ultra-violet and transmitting an average of about 60 per cent. of that throughout its range, was also used, instead of the quartz glass cover,

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with both the larvae and egg masses, exposed to sunlight and to the mercury vapor lamp. The object of using this dark glass was to test whether or not ultra-violet, alone as a factor, is capable of bringing about lethal effects upon the nematodes.

Experimental. Before exposing the larvae and eggs to the sun, elimination of the heat of sunlight as an injurious factor had to be considered. The larvae and eggs were exposed on separate drops of water about ½ mm. deep on slides placed in Petri dishes lined with moist filter paper. The constant addition of water to the drop on the slide prevented the water from heating up to a temperature higher than 30° C. Not a single nematode subjected to a temperature of 35° C. for 4 hours in a separate experiment on heating effects was killed. Hence, the drop of water and also the low temperature eliminated the heat and drying factors. Simultaneous exposure under identical conditions with regular glass Petri dish covers, instead of quartz covers, showed no killing of larvae even after several hours, which would seem to eliminate heat as a factor, since glass is penetrable to heat but not to ultra-violet.

As a still further check on the temperature factor, a series of 3 slides, each containing 100 fresh, active larvae in a drop of water, was placed upon a flat tray of ice and exposed to the sunlight for the same periods of time. The results in percentage killed were not perceptibly different from those obtained from the usual method of exposure. Separate tests had shown that actual freezing of the water containing Heterodera larvae over ice and salt did not kill them, even when freezing was repeated several times with the same lot of material. Cold, therefore, was not a factor in killing.

Exposures in Petri dishes without covers as compared with those with quartz glass covers gave no perceptible difference with regard to the effect of the ultra-violet rays. Several preliminary experiments determining this point were carried out with approximately the same results. This is to be expected since quartz is completely transparent to solar radiation.

The series of experiments was repeated 6 times. The average results obtained are presented in tables 4 and 5.

Subjection of larvae to ultra-violet radiation by exposure to sunlight for 25 minutes killed all the larvae. Irradiation by the mercury vapor lamp killed them after exposure for 12 minutes. With eggs, a longer time was required to produce any killing effect. When exposed to sunlight it took 5 hours, and when exposed to light from a mercury vapor lamp, 4 hours to kill every egg in the egg mass. The time required for killing free eggs, however, was considerably shorter. It would seem definitely that the material of which the gelatinous matrix of the egg mass is composed serves to some degree as a protection against the penetration of ultra-violet rays.

TABLE 4.—*Effect of different periods of exposure to light on the life of larvae*

Time of exposure		Percentage killed			
		Exposure to sunlight		Exposure to light from mercury vapor lamp	
Hours	Minutes	Under quartz glass	Under dark glass	Under quartz glass	Under dark glass
	1				
	3			0	
	5	0		3	
	7	1		14	
	10	3		72	
	12	5		100	
	15	8		100	
	20	14			
	25	100			
	30	100	0		0
	40		0		6
	45		15		60
1			38		100
1	30		100		100
2			100		
Controls		0	0	0	0

Irradiation both by the mercury vapor lamp and the sunlight under the dark Corex red purple glass required a considerably longer time to produce killing effects on the larvae as well as on the eggs. This is only to be expected in view of the fact that, while this special glass transmits only ultra-violet, it transmits incompletely in intensity the ultra-violet spectrum. Quartz glass transmits without loss in intensity practically all solar radiation. The literature on biological effects of light indicates, however, that aside from those rays that are actually transformed to heat (controlled here) it is only the ultra-violet that produces lethal effects. This makes it even more evident that ultra-violet, alone, is capable of producing the lethal effects on Heterodera, evident from these tests. From tables 4 and 5 it is possible, by referring across from the first 100 in each column to the time column, to see at a glance the minimum time tested, required for complete killing. The correct time for this is, of course, somewhere between this figure and the next shorter period.

It is true that the sunlight that reaches the surface of the earth is different not only in various localities but also in the same localities on different days and at different times of the day. There are very striking

TABLE 5.—*Effect of different periods of exposure to light on egg viability*

Time of exposure		Percentage killed				
		Exposure to sunlight			Exposure to light from mercury vapor lamp	
Hours	Minutes	Under quartz glass		Under dark glass	Under quartz glass	Under dark glass
		Eggs in masses	Eggs free	Eggs in masses	Eggs in masses	Eggs in masses
	10		25.3			
	20		28.4		77.67	
	30	10.84	30.0		80.0	
1		16.23	33.8		95.0	
1	30	20.6	100.0		95.7	
2		34.7	100.0		96.3	
2	30	47.2			98.0	
3		61.25			99.4	
4		65.0			100.0	
5		100.0			100.0	
6		100.0		95.2		99.3
7				99.8		100.0
8				100.0		100.0
9				100.0		

differences in insolation even on successive days that seem similar in every respect. Freer (7) pointed out that greater intensities of ultra-violet rays may sometimes occur on hazy days than on days that are bright and clear. This finding was corroborated by Lois Godfrey (11) for Hawaii conditions.

To obtain a quantitative estimate of the intensity of ultra-violet, a solution of N/10 oxalic acid and N/100 uranyl sulphate was subjected to the action of the sunlight according to the method of Anderson and Robinson (1) used by Lois Godfrey (11) in Hawaii. The rate of decomposition of the oxalic acid by the solar radiation in the presence of uranyl sulphate is directly proportional to the intensity of the ultra-violet rays. Ten cubic centimeters of the solution were pipetted into a quartz-cover dish and exposed to the sunlight for 30 minutes. A new dish was brought out at every 30-minute period throughout the length of any particular long-time exposure. The solutions after exposure were titrated with N/10 potassium permanganate and the amount of oxalic acid decomposition calculated. The intensity of ultra-violet is indicated by the amount of decomposition produced in the oxalic acid. Table 6 gives the results of tests in which killing effect on nematodes was measured simultaneously with a measurement of intensity of ultra-violet.

TABLE 6.—*Effect of various intensities of ultra-violet radiation as indicated by oxalic acid decomposition on the life of Heterodera larvae*

Time of exposure in minutes	Time of day					
	8: 45–9: 15 a. m.		11: 00–11: 30 a. m.		3: 30–4: 00 p. m.	
	Oxalic acid percentage of decomposition	Percentage killed	Oxalic acid percentage of decomposition	Percentage killed	Oxalic acid percentage of decomposition	Percentage killed
15	64.4	4	71.1	7	51.0	3
20		18		100		11
25		100		100		100
30		100		100		100
	Time of day					
	9: 10–9: 40 a. m.		11: 10–11: 40 a. m.		2: 20–2: 50 p. m.	
15	55.4	4	43.0	3	82.0	47
20		12		8		100
25		100		100		100
30		100		100		100

At the end of 24 hours, all the larvae on the slides, which served as controls, were alive and active. The controls bearing egg masses for all ultra-violet tests, after a period of 24 days, all showed a consistently high percentage of hatching. (Average 98.1 per cent.)

From these observations, it is evident that irradiation with ultra-violet has a lethal effect on both the larvae and eggs of *Heterodera radicicola.* Also, the prolongation of the time of exposure increases the killing effect. The data, furthermore, indicate that the greater the intensity of ultra-violet, the shorter the time required for killing. This latter observation is strikingly shown in table 6. In the exposures on April 23rd, when ultra-violet intensity was high at noon, as manifested by oxalic acid decomposition of 71.1 per cent. in ½ hour, all the larvae were killed in 20 minutes. With the lower intensities of both morning and afternoon, killing was definitely delayed. On April 24th, when, because of cloudiness, the light intensity near noon was cut down (only 43.0 per cent. decomposition), the killing effect was greatly delayed. In the afternoon in bright sunlight, giving 82.0 per cent. decomposition, killing was hastened again.

EFFECT OF COMBINED EXPOSURE TO SUNLIGHT AND DRYING ON THE LIFE OF LARVAE AND ON EGG VIABILITY

Since the investigations have proved that nematodes can be killed by any one of the 3 factors—light, heat (13), and drying, alone—it would ap-

pear that the simultaneous treatment with sunlight and drying would produce lethal effects more rapidly. There is the probability of further reduction of the time by the heat factor.

One hundred fresh, active larvae were placed in a drop of water on each slide and left in a shady place in the laboratory for preliminary drying. As soon as the slide looked dry to the unaided eye it was examined for water under a microscope. If no free water was detected and if the nematodes were all still moving about, the slide was immediately placed on the window sill upon which the sunlight shone directly. The egg masses also were exposed to the sunlight under dry conditions. After the completion of each test they were placed under favorable conditions for hatching to determine the effect on egg viability.

TABLE 7.—*Data showing the combined effect of exposure to sunlight and drying on the life of the larvae and on egg viability*

Time of exposure	Percentage killed		
	Larvae	Eggs in masses	Eggs free
15 sec.	0		
30 "	31		
45 "	60		
1 min.	93	14.0	15.2
2 "	100		27.4
3 "	100	16.1	30.0
5 "	100	17.6	52.4
10 "		23.0	70.4
15 "		69.6	93.8
20 "		98.4	100.0
25 "		99.0	
30 "		100.0	
40 "		100.0	

Larvae were exposed to the sunlight in a dry condition for various periods from 15 seconds to 5 minutes. The eggs were exposed for different periods from 1 minute to 1 hour. Table 7 shows that all the larvae were killed after an exposure of $1\frac{1}{2}$ minutes $\pm$ $\frac{1}{2}$ minute; the eggs in egg masses, after $27\frac{1}{2}$ minutes $\pm$ $2\frac{1}{2}$ minutes; eggs free, $17\frac{1}{2}$ minutes $\pm$ $2\frac{1}{2}$ minutes. The results produced by the sunlight, drying, and heating show a striking combined effect in rapid killing of the nematodes in all stages. Figure 4 shows interesting straight-line relationship between time and killing effect of the sun, depicted on a semilog graph. The relative rates of killing on exposure to the sun are clearly shown here.

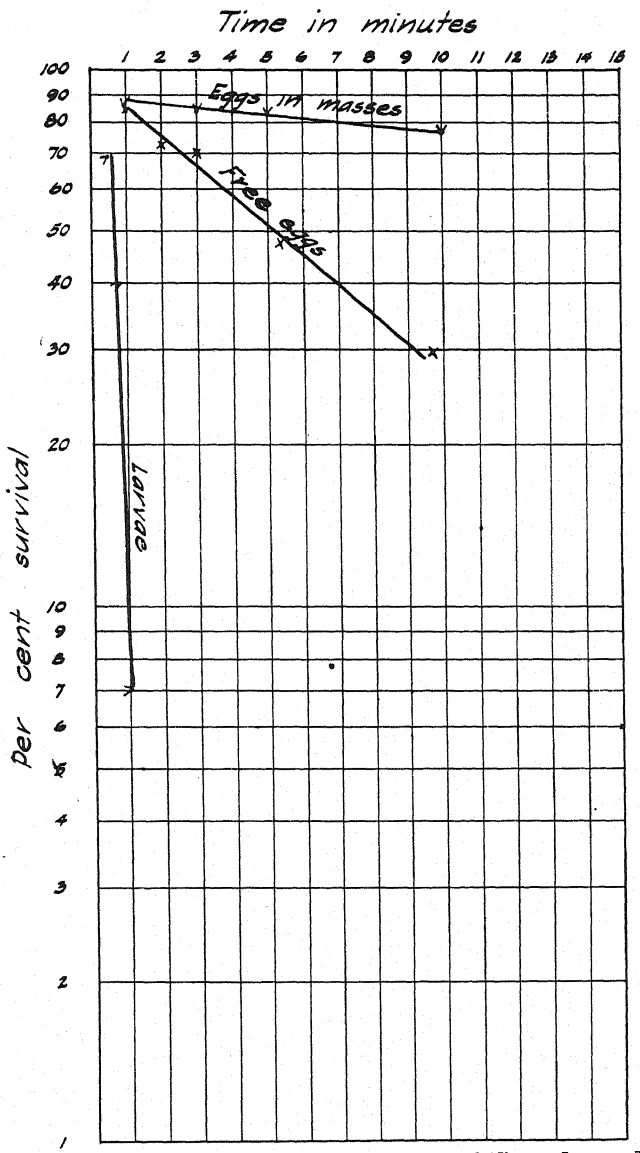


FIG. 4. Semilog graph showing percentage survival of *Heterodera radicicola* larvae and eggs, at different intervals, when exposed to the direct action of sunlight, sun heat, and sun drying. The outstanding feature of this graph is the high resistance of eggs protected in their gelatinous matrix, as compared with the free eggs and larvae. The free eggs are, likewise, considerably more resistant than the larvae.

At the end of 24 hours control slides showed all the larvae to be alive. Egg masses placed as checks, according to the usual procedure, gave hatching of 97.3 and 98.7 per cent.

Another test was made with egg masses covered with a very thin layer of soil (about 1½ mm.) to get some indication, if possible, as to the extent to which the killing effect of ultra-violet penetrated through such a medium. In one set, moist conditions were maintained, the soil being kept moist and exposure to midday sun being in a Petri dish covered with quartz glass. There were 3 replications of treatments. In this case, average percentages of survival were as follows: 30 minutes' exposure, 96.4; 1 hour, 91.3; 2 hours, 84.8; 4 hours, 7.3; 6 hours, 0.9; 7 hours, 0. Complete killing in this case, in 6½ hours ± ½ hour, is to be compared with 4½ hours ± ½ hour, where egg masses were exposed directly, under similar conditions (ignoring the possibility of great differences in sunlight intensity on the 2 days). In another set, the eggs were covered with air-dry soil and exposed directly to the midday sun. Here, of course, drying is a factor, in addition to ultra-violet. Here complete killing was attained in 1¼ hours ± ¼ hour, which is to be compared with 27½ minutes ± 2½ minutes in the case of direct exposure of egg masses, without soil, on another day. Certainly in both tests marked insulation on the part of the soil against lethal effects of sunlight is manifested.

EFFECT OF EXPOSURE OF ROOT GALLS TO DIFFERENT PERCENTAGES OF RELATIVE HUMIDITY AND TO SUN DRYING

Preliminary observations and experience having shown that *Heterodera radicicola* as contained within more or less woody roots of plants is protected against unfavorable environmental conditions, a final test was made to determine approximately the extent to which such protection acts. For this purpose typical nematode root galls were selected from cowpea plants and from pineapple plants. The cowpea galls were on relatively young roots and consequently were more or less soft and succulent. Some of the contained egg masses were external, as illustrated in the senior writer's paper (9) on technique. The pineapple galls were mostly terminal and therefore more or less succulent at the very tip but were much more woody, in general, than the cowpea galls, and the egg masses were entirely internal.

Such galls were washed and dried on paper towels and left for a few minutes to become thoroughly dry on the surface. They were then exposed, as described for larvae and eggs, by means of desiccators, to 50, 60, 70, 80, and 90 per cent relative humidity, to complete dryness (over concentrated sulphuric acid), and to direct sunlight, all for varying periods. Immediately after the completion of each test the roots were dissected to re-

TABLE 8.—*Percentage survival of Heterodera radicicola in root galls of cowpea (c) and pineapple (p) exposed to different relative humidities*

Time of exposure, days	1		2		3		4		5		6	
Relative humidity (per cent)	c	p	c	p	c	p	c	p	c	p	c	p
50	86.2	91.0	0	18.0	0	0	0	0				
60	97.3	98.5	95.6	94.7	94.9	95.9	24.9	32.8	0	0	0	0
70	97.5	98.7	97.5	97.4	96.9	97.0	26.6	49.2		8.0	0	0
80	98.8	98.1	98.7	98.9	97.8	97.1			75.7	73.5		
90	99.1	99.2	98.9	99.2	99.1	99.2						
100												
0	0	52.2	0	0	0	0						
Sunlight	8 hr.		16 hr.		24 hr.		30 hr.					
	0	52.1	0	2.0	0	0						

Time of exposure, days	7		8		9		10		15		20	
Relative humidity (per cent)	c	p	c	p	c	p	c	p	c	p	c	p
50												
60												
70	0	0										
80	33.8	39.5	0	0								
90	96.8	97.3	74.8	84.1			74.3	79.8	49.9	69.0	49.2	69.7
100							82.6	89.3	74.8	83.4	69.2	74.3
0												

move contained egg masses, which were mounted on slides in the usual method for incubation at room temperature.

The series of tests was repeated 3 times, with 1 root of each kind in each test. Results were surprisingly uniform, considering the great variability of protecting root material surrounding the eggs. Results in averages of the 3 tests, only, are given here, in tabulated form, in table 8. Control roots, in which the egg masses were dissected out of fresh roots and incubated directly without exposure, gave a uniformly high rate of viability of eggs, over 99 per cent. in both cowpea and pineapple roots.

It is possible to see at a glance the important conclusions that are to be drawn from this test. The viability of eggs is maintained for a much longer period when they are protected by the root than when free. At 50 per cent relative humidity, for example, eggs in roots survive for over a day in the case of cowpea and over 2 days in woody pineapple roots, as compared with only about 2 hours in freed egg masses and only 1 hour as free eggs. With higher humidities, life is maintained for a longer period. At 80 per cent humidity, all were killed by the 8th day, while at 90 and 100 per cent there was still a high proportion of living eggs at the end of the 20th day. There was a gradual decrease in numbers of living eggs, and one is justified in assuming that drying would eventually become complete at 90 per cent humidity, with consequent death of the eggs. In our tests, after prolonged exposure at the higher humidities, the roots became moldy and gradually decomposed, thus introducing an external uncontrolled factor.

With complete drying and with exposure to direct sunlight, killing was, likewise, much slower in the root galls than with free eggs and egg masses. Killing was complete, however, in less than 24 hours in both cases, somewhat faster in the cowpea than in the pineapple galls. Sunlight exposure killed the nematodes in the cowpea roots in less than 8 hours. The well-known fact of low penetrating power of ultra-violet should be noted here in explanation of the much-slower killing of eggs in roots than of eggs exposed directly.

SUMMARY AND CONCLUSIONS

As a first step in the study of environmental relations of the root-knot nematode, *Heterodera radicicola,* a large number of egg masses were placed under favorable conditions for hatching, in drops of fresh tap water on slides in moist chambers, at room temperature (24 to 28° C.). The average hatch from 84 egg masses was 97.8 per cent. The average number of eggs per egg mass was 416.

In the study of effects of different percentages of relative humidity (as obtained over sulphuric acid of controlled densities, in desiccators) on

longevity of larvae and eggs, the following results were obtained: at 50 per cent humidity, larvae survived for 3½ minutes ± ½ minute; eggs in masses for 2¼ hour ± 15 min; free eggs for 1¼ hours ±15 minutes; eggs as protected by root-gall tissues for 1½ days ± ½ day. At 90 per cent humidity, larvae survived for 25 minutes ± 5 minutes; eggs in egg masses for 8½ hours ± ½ hour; free eggs for 5½ hours ± ½ hour; eggs in root-gall tissues, 75 per cent still alive after 20 days' exposure. For intermediate humidities, periods of survival were intermediate. One hundred per cent humidity kept all stages alive throughout the course of the experiment (or until molds and putrefaction killed them). Complete dryness brought about killing as follows: larvae, in 2½ minutes ± ½ minute; eggs in egg masses in 1¾ hours ± ¼ hour; free eggs in 52½ minutes ± 7½ minutes; eggs in root-gall tissues in 1½ days ± ½ day.

Ultra-violet radiation was, likewise, found to have a relatively quick lethal effect on this nematode in different stages. The ultra-violet of the midday sun on a clear day in April in Hawaii, passing through quartz glass covers, killed the different stages in drops of water at nonlethal temperatures as follows: larvae in 22½ minutes ± 2½ minutes; eggs in egg masses in 4½ hours ± ½ hour; free eggs in 1¼ hours ± ¼ hour. Killing with the ultra-violet of a Cooper-Hewitt air-cooled quartz mercury vapor lamp at a distance of 50 cm. was always in a shorter period than in sunlight. Measurement of the ultra-violet made at the time of some of the tests showed killing was hastened with increased intensity of ultra-violet.

Killing of the nematodes from the combined effects of sunlight, heat, and drying took place in a remarkably short time. Larvae were killed in 1½ minutes ± ½ minute; eggs in egg masses in 27½ minutes ± 2½ minutes; free eggs in 17½ minutes ± 2½ minutes; eggs in root-gall tissues of cowpea in less than 8 hours and in the more woody tissues of pineapple roots in about 16 hours (8-hour periods on 2 successive days).

Analysis of percentage survival of larvae and free eggs for different periods shorter than the lethal period, for many of the different kinds of treatments, showed an interesting straight-line relationship between time and percentage survival, as graphed on semilog paper.

UNIVERSITY OF HAWAII,
HONOLULU, HAWAII.

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PHYSALOSPORA ZEICOLA ON CORN AND ITS TAXONOMIC AND HOST RELATIONSHIPS

A. H. Eddins and R. K. Voorhees

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INTRODUCTION

In an earlier paper the senior writer (1) reported that *Diplodia frumenti* E. and E., *D. natalensis* Evans, *D. gossypina* Cke., and *D. tubericola* E. and E. cause similar ear rots of corn in Florida. Since the morphological characters of the imperfect stages of these 4 organisms also are similar, the only means left to determine whether they are the same or different species is to compare their perfect stages. Stevens (8) has shown that *D. natalensis* and *D. gossypina* are synonymous, as they have *Physalospora rhodina* (Berk. and Curt.) Cooke as their perfect stage; but no one has yet reported the perfect form of either *D. frumenti* or *D. tubericola.*

In 1929 a species of Physalospora was found on cornstalks in Florida and studies were undertaken to determine its pathogenicity and identity and to determine whether it is the perfect stage of *Diplodia frumenti.* In the course of these studies, information was obtained on the comparative susceptibility of ears of corn in different stages of development to the fungus. Also, a number of different plant species were inoculated with this Physalospora, *P. rhodina,* and *D. tubericola* to obtain some information as to their comparative host range. Results of these investigations are given in this paper.

CAUSAL ORGANISM

The first specimens of the species of Physalospora herein considered were collected in the spring of 1929 on old dead cornstalks in fields in the vicinity of Melrose and Gainesville, Florida. Since that time mature asci and ascospores were usually found in late summer and fall on the nodes and internodes of the culm near the base of the plant after the infected stalks had dried.

Five ascospore cultures of this Physalospora were grown on various kinds of media. Two of these produced pycnidia in potato-dextrose agar and sterilized corn meal, which contained pycnospores typical of those produced by *Diplodia frumenti.* The same 2 cultures also formed pycnidia on the inoculated stems of geranium plants that were growing in the greenhouse. The other 3 cultures failed to fruit on media and geranium stems. Cultures obtained from either ascospores or pycnospores of this Physalospora and from pycnospores of the species of Diplodia fruited more readily

on artificial media immediately after they were isolated than after they had been carried in culture for several months. Old cultures, however, were pathogenic, produced spores on the host plant, and when reisolated sporulated in culture.

Pathogenicity. In July, 1931, green cornstalks that had ceased to elongate were inoculated with 5 cultures of this Physalospora by placing the mycelium in an incision made by a downward stroke with a scalpel above the 4th or 5th node and wrapping the wound with nurseryman's tape. Notes on infection (Table 1) were taken 6 weeks later. The pith and epidermis of the infected stalks were blackened in the zone invaded by the fungus for several inches on either side of the point of inoculation. Three of the cultures formed the perfect stage on some stalks and the imperfect on others, while 2 produced the imperfect stage only. On some diseased stalks no fruiting bodies were formed, but stromata of the fungus were present on the surface in the infected zone. The perithecia, asci, and ascospores were similar morphologically to those of the Physalospora from which the cultures used in the inoculations were obtained, and the pycnidia and pycnospores were like those of *Diplodia frumenti,* which is common on corn in Florida.

TABLE 1.—*Results obtained by inoculating cornstalks with mycelium from ascospore cultures of the Physalospora from corn*

Culture	Number of stalks				
	Inoculated	Infected	With perithecia alone	With pycnidia alone	With stromata containing no spores
1	27	27	19	2	6
2	25	25	0	25	0
3	25	25	0	25	0
4	25	25	13	4	8
5	25	23	8	13	2
Check	25	2	0	2	0

Ears of corn in the early-milk stage were inoculated by inserting mycelium from pure cultures of the Physalospora into wounds made in the shanks, and the results are given in table 2. Many of the ears were invaded and thoroughly rotted and blackened by the fungus, while in others the infection was scattered over the ear (Fig. 1, A). The rot produced by this organism was similar to that caused by *Diplodia frumenti* (1). The fungus produced perithecia on the husks of some ears and pycnidia on others.

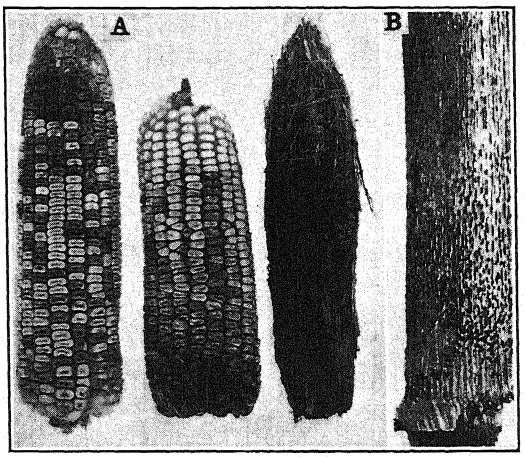


FIG. 1. A. Ears of corn showing kind of rot produced by artificial inoculation, with mycelium developing from an ascospore of *Physalospora zeicola*. B. Perithecia of *P. zeicola* breaking through the epidermis of a cornstalk.

TABLE 2.—*Results obtained by inserting mycelium of ascospore cultures of the Physalospora from corn into the shanks of ears in the early-milk stage of development*

Culture	Number of ears	
	Inoculated	Infected
1	22	12
2	25	10
3	23	18
4	24	19
5	23	10
Check	24	1

Taxonomy. In 1890 Ellis and Everhart (3) described a species of Physalospora on cornstalks and named it *Physalospora zeicola* E. and E., the original description of which is as follows:

"*Physalospora zeicola* E. and E. On dead stalks of Zea mays exposed to the weather through the winter, Newfield, N. J. Apr. 1890. Perithecia gregarious, minute ($\frac{1}{4}$–$\frac{1}{3}$ mm) covered by the cuticle which is slightly raised and pierced by the obtusely conical, black and shining ostiolum. Asci clavate-cylindrical, 75–80 × 12–15 μ, nearly sessile, with abundant paraphyses, sporidia crowded-biserate, elliptical or almond shaped, hyaline, granular, 18–20 × 8–10 μ."

The perithecia of the Physalospora on cornstalks in Florida are similar to those of *Physalospora zeicola* in that they are black, gregarious, and break

through longitudinal cracks in the epidermis (Fig. 1, B). The Florida fungus also forms its perithecia in stromata on the surface of the culm at the nodes.

The asci of the Florida Physalospora have the same shape but are longer than those of *P. zeicola,* the measurements of the former being 95–140 × 10–13 μ. Also, certain characteristics of the asci that were not mentioned in Ellis and Everhart's (3) description of *P. zeicola* were observed in the Florida Physalospora. The young ascus has its granular contents enclosed in a sac that lies in the space enclosed by the outer wall of this structure (Fig. 2, C). After the spores are formed and mature the wall of the sac

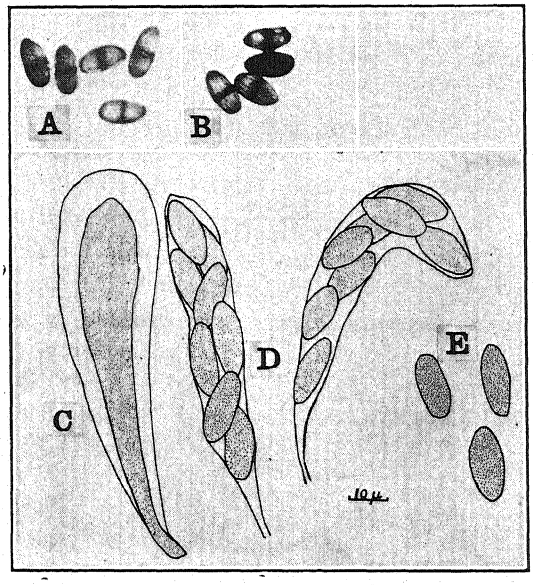


FIG. 2. A and B. Photomicrographs of pycnospores from: A, *Diplodia frumenti;* B, *Physalospora zeicola.* C–E. Drawings of asci and ascospores of *P. zeicola:* C, young ascus; D, mature asci; E, mature ascospores.

remains to enclose the spores, while the outer wall disappears (Fig. 2, D). When cut across the asci have a white appearance that, according to Stevens (8), is characteristic of the genus Physalospora.

Ascospores of the Florida Physalospora (Fig. 2, E) are of the same shape and are unicellular, hyaline, and granular, like those of *P. zeicola.* Two hundred ascospores of the former measured 13–27 × 6–11 μ, mostly 20–23 × 8–9 μ, thus agreeing very closely in size with those of *P. zeicola.*

Physalospora zeicola differs from *P. rhodina* in that it has shorter and narrower ascospores, as those of the former measure 13–27 × 6–11 μ, mostly 20–23 × 8–9 μ, while those of the latter, according to Stevens (8), measure

24–42 × 7–17 μ, mostly 30–35 × 11–14 μ. Stout (9) reported that asci of *P. zeae* Stout measure 85–150 × 13–22 μ and the ascospores 19–25 × 6.5–8 μ and concluded that it was a species distinguished from *P. zeicola* because of its larger asci and longer but narrower spores. It thus appears that the morphological characters of the Florida Physalospora are, in general, very similar to those given for *P. zeicola*, as indicated in the above comparisons, and it is referred to that species.

On potato-dextrose agar an ascospore culture of *Physalospora zeicola* cannot be distinguished from a pycnospore culture of *Diplodia frumenti*. The young mycelium of each is hyaline, granular, septate and branched, producing a loose, flocculent, white aerial growth. When the cultures grow older, the mycelia become brown and stromatic masses and pycnidia are formed on the surface of the media. On severely diseased ears cultures from each source form brown, felty masses of hyphae on the kernels and husks.

The imperfect as well as the perfect stage of *Physalospora zeicola* developed on cornstalks artificially inoculated with mycelial cultures derived from ascospores of this fungus. The morphological characters of the pycnidia and pycnospores observed on these infected stalks were similar to those occurring on stalks naturally and artificially inoculated with *Diplodia frumenti*. Pycnospores of *P. zeicola* from artificially inoculated stalks measured 21–29 × 10–14 μ, mostly 25–27 × 12–13 μ, and from culture 21–33 × 12–15 μ, mostly 26–28 × 13–14 μ; and those of *D. frumenti* from naturally infected stalks measured 19–31 ×11–15 μ, mostly 25–27 × 13–14 μ, and from culture 20–28 × 11–15 μ, mostly 24–26 × 13–14 μ (Tables 3 and 4).

TABLE 3.—*Classification of pycnospores developing from the perfect and imperfect stages of Physalospora zeicola according to length*

Source of culture	Total number spores	Number of spores having a given length in microns													
		19	20	21	22	23	24	25	26	27	28	29	30	31	33
Physalospora zeicola from cornstalks	100			2	2	5	9	26	22	25	6	3			
Physalospora zeicola from culture	100			2	1	11	3	14	23	15	15	11	6		1
Diplodia frumenti from cornstalks	100	1	5	7	15	20	18	44	24	28	19	13	4	2	
Diplodia frumenti from culture	100		1	5	8	14	24	21	18	4	5				

TABLE 4.—*Classification of pycnospores developing from the perfect and imperfect stages of Physalospora zeicola according to width*

Source of culture	Total number spores	Number of spores having a given width in microns					
		10	11	12	13	14	15
Physalospora zeicola from cornstalks	100	3	15	38	28	16	
Physalospora zeicola from culture	100			9	33	39	19
Diplodia frumenti from cornstalks	100		10	28	82	61	19
Diplodia frumenti from culture	100		3	7	55	29	6

Thus, the imperfect spores from the 2 sources agree closely in size.

A small portion of a specimen of *Diplodia frumenti* on a cornstalk collected by Langlois in Louisiana in 1886 was obtained from the U. S. Herbarium, Washington, D. C. Twenty-six pycnospores found on this material measured 17–26 × 10–15 μ, thus agreeing very well in size with pycnospores of *Physalospora zeicola* and *D. frumenti,* reported above, but are somewhat larger than the measurements reported by Ellis and Everhart (2) in their original description of *D. frumenti* in which the dimensions were given as 15–18 ×12 μ.

Since the imperfect stage of *Physalospora zeicola* cannot be distinguished from *Diplodia frumenti* in its growth on media in pure culture and on cornstalks and ears and since both form pycnidia in the same manner, containing pycnospores of similar shape, color, septation, and approximately the same dimensions, *P. zeicola* is considered to be the perfect stage of *D. frumenti.* Photomicrographs of pycnospores of *D. frumenti* and *P. zeicola* are shown in figure 2, A and B.

COMPARATIVE SUSCEPTIBILITY OF EARS OF CORN AT DIFFERENT STAGES OF GROWTH

Experiments were conducted in the field in which ears of corn of different stages of growth were inoculated by different methods. The inoculum consisted of pycnospores and mycelium from pycnospore cultures of *Physalospora zeicola.* At intervals of 5 to 9 days from June 10 to July 9, inclusive, 17 to 35 ears were inoculated by each of 4 methods, as follows: (1) Approximately 10 cc. of a spore suspension sprayed on ear tips; (2) approximately 10 cc. of a spore suspension sprayed in the crevice between the ear and sheath; (3) approximately 10 cc. of a spore suspension injected

into the husks near the butt end with a hypodermic syringe; (4) a corn kernel on which the fungus was growing was inserted in the shank of each ear between the butt end of the ear and its point of attachment to the stalk.

The inoculated ears were harvested August 18 and examined for visible signs of infection, as indicated by the blackened condition of diseased kernels. As shown in table 5, infection was obtained by all 4 methods of inoculation. The method producing the highest percentage of infected ears with visible signs of rotting was that in which an infected corn kernel was inserted in the shank. Injecting a spore suspension into the husks gave a higher rate of infection than was obtained by spraying the inoculum on the tips of the ears and in the crevices between the ears and sheaths. The fact that inoculations made by wounding the ears gave higher rates of infection than those made without wounding was probably due to the possibility that the spores injected into the ear husks and the mycelium inserted in the moist shanks suffered less from lack of moisture than the spores sprayed on the tips and butts of the ears.

TABLE 5.—*Comparative susceptibility to Physalospora zeicola of corn ears in different stages of development*

Date of inoculation	Method of inoculation	Stage of development of ears	Total ears inoculated	Ears with rotted kernels	Ears with pycnidia on husks
June 10	Spore suspension sprayed on ear tips	Blister	23	3	8
" 17	"	Early milk	22	3	15
" 22	"	Milk	23	2	7
" 30	"	Late milk	25	1	3
July 9	"	Dent	25	0	0
June 10	Spore suspension sprayed in crevices between ear and sheath	Blister	25	3	14
" 17	"	Early milk	23	3	14
" 22	"	Milk	17	0	4
" 30	"	Late milk	24	2	2
July 9	"	Dent	25	0	1
June 10	Spore suspension injected into husks near the butt	Blister	25	9	21
" 17	"	Early milk	25	4	16
" 22	"	Milk	lost	.....	.....
" 30	"	Late milk	25	5	6
July 9	"	Dent	26	0	5
June 10	Inoculum inserted in ear shank	Blister	35	9	33
" 17	"	Early milk	17	7	14
" 22	"	Milk	27	15	21
" 30	"	Late milk	24	5	13
July 9	"	Dent	25	2	0

Ears in the early stages of growth were more susceptible to infection than those in the later stages of development. This was probably because the young, tender ears were easily penetrated and contained more moisture to support the growth of the fungus than older ears that were beginning to dent and harden. The fungus produced pycnidia on the husks of some ears that showed no kernel infection as well as on those whose kernels showed signs of rotting.

Under natural conditions more ears are infected by *Physalospora zeicola* at the butt than at the tip. Such is also true of ears naturally infected with *Diplodia zeae* (Schw.) Lev., according to Raleigh (6). Ears inoculated at the tip with a pycnospore suspension of *P. zeicola* had the inoculum forced on them in a stream from a pressure sprayer, thus placing the spores well down between the silks and in the crevices between the tips of the husks where moisture conditions were suitable for germination and fungus growth. Under natural conditions spores falling on the tips of ears seldom meet with conditions suitable for germination (6).

HOSTS OF PHYSALOSPORA ZEICOLA, PHYSALOSPORA RHODINA AND DIPLODIA TUBERICOLA

Taubenhaus (10) reported that *Diplodia natalensis* and *D. gossypina,* which, according to Stevens (8), are synonymous names for the imperfect stage of *Physalospora rhodina,* caused the typical Java black rot of sweet potatoes. Meier (5) found that *D. tubericola* from sweet potato would cause the stem-end root of watermelon; and Fawcett and Burger (4) discovered that Diplodia isolated from gumming peach trees caused a gumming of orange trees and Diplodia isolated from orange trees caused a gumming of peach trees. Cross-inoculation studies made by Eddins (1) showed that *D. tubericola, D. natalensis,* and *D. gossypina* caused a rot of ears of corn similar to that caused by *D. frumenti,* the imperfect form of *P. zeicola,* and each caused a similar rot of oranges, grapefruit, sweet potatoes, cotton bolls, and watermelons. In order to get some idea as to the extent of the host range of *P. zeicola, P. rhodina,* and *Diplodia tubericola,* 31 species of woody and herbaceous plants representing 24 families were inoculated with mycelium from pure cultures originating from pycnospores of the imperfect stages of the 3 organisms.

From 5 to 10 inoculations were made in the branches, stems, and other parts of the different plants. A branch or stem inoculation was made by inserting mycelium of the fungus scraped off the surface of potato-dextrose agar into a small wound made in the bark or outer layer of tissues with a sharp knife. After inoculation the wound was first covered with waxed paper and then wrapped with nurseryman's tape. Branch or stem inoculations were made in all plants of the different species except *Citrullus vulgaris* Schrad. (watermelon) and *Gossypium hirsutum* L. (cotton), in which

the fruits alone were inoculated, and *Ipomoea batatas* (L.) Lam. (sweet potato), in which the fleshy roots were inoculated. A watermelon was inoculated by sponging it off at the stem end with 50 per cent alcohol, and placing mycelium of the fungus in the bottom of a small plug cut into the stem, after which the plug was replaced and the wound was covered with waxed paper and nurseryman's tape. Green cotton bolls and sweet potatoes were inoculated in the same manner as watermelons except that their entire surfaces were sterilized before inoculation and inoculum was inserted in wounds at any convenient point on the surfaces of the bolls and roots.

The inoculation experiments were carefully checked by wounding branches, stems, and other parts of the different plants and treating them similarly to those that were inoculated except that inoculum of the different fungi was not introduced into the wounds.

The inoculations were made in the summer of 1930 and 1931 and the criterion for infection was the production of pycnidia and mature pycnospores in the region killed by the Physalospora or Diplodia in inoculated areas on the plants. From 1 to 6 weeks was required for these organisms to produce pycnidia on their hosts. A longer time was required for the

TABLE 6.—*Host plants of Physalospora zeicola, P. rhodina, and Diplodia tubericola as determined by the production of pycnidia and pycnospores following artificial inoculation*

Family	Species	Family	Species
Aceraceae	*Acer floridanum* Thumb.	Magnoliaceae	*Magnolia grandiflora* L.
Amygdalaceae	*Amygdalus persica* L.	Malaceae	*Pyrus communis* L.
	Prunus angustifolia Marsh.	Malvaceae	*Gossypium hirsutum* L.
Artocarpaceae	*Ficus carica* L.	Meliaceae	*Melia azedarach* L.
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	Mimosaceae	*Acacia macrantha* Thumb.
Cucurbitaceae	*Citrullus vulgaris* Schrad.	Papayaceae	*Carica papaya* L.
Ebenaceae	*Diospyros virginiana* L.	Pinaceae	*Pinus caribaea* Morelet
Euphorbiaceae	*Aleurites fordii* Hemsl.	Poaceae	*Zea mays* L.
	Euphorbia pulcherrima Willd.	Proteaceae	*Grevillea robusta* A. Cunn.
	Ricinus communis L.	Rutaceae	*Citrus maxima* (Burm.) Merrill
Fabaceae	*Crotalaria striata* DC.		*Citrus nobilis* Lour. var. *unshiu* Swingle
Fagaceae	*Quercus laurifolia* Michx.		*Citrus sinensis* Osbeck
Geraniaceae	*Pelargonium hortorum* Bailey	Spondiaceae	*Mangifera indica* L.
Hamamelidaceae	*Liquidambar styraciflua* L.		
Juglandaceae	*Hicoria glabra* (Mill.) Britt.		
Lauraceae	*Camphora camphora* (L.) Karst.		
	Persea persea (L.) Cockerell		
	Sassafras sassafras (L.) Karst.		

fungus to fruit on the woody plants than on the herbaceous ones. They usually fruited within a week to 10 days on watermelons, while on cotton bolls 2 to 3 weeks were needed and on sweet potatoes a month to 6 weeks were required. No difference was observed in symptoms produced on the different hosts by the 2 species of Physalospora and *D. tubericola*. None of the check inoculations in the different plants showed any infection from Diplodia. A list of the host plants, as determined by these inoculation experiments, is given in table 6.

SUMMARY

A Physalospora found on cornstalks in Florida has been identified as *Physalospora zeicola* E. and E., and it has been demonstrated that it produces an ear rot similar to that caused by *Diplodia frumenti*.

Data are presented to show that *P. zeicola* is the perfect stage of *D. frumenti*, and the fungus is referred to in this paper by its perfect-stage name. Since Stevens (8) has shown that *P. rhodina* is the perfect stage of *D. natalensis* and *D. gossypina*, it would be less confusing to refer to the fungus by its perfect-stage name than to call it *D. natalensis* when it appears on citrus and *D. gossypina* when found on cotton.

Ears of corn in the early stages of growth proved more susceptible to infection with *P. zeicola* than ears in the later stages of development.

Physalospora zeicola, *P. rhodina*, and *Diplodia tubericola* grew and produced pycnidia on 31 host plants inoculated with mycelium from pure cultures obtained from pycnospores of the imperfect stages of the 3 organisms.

These studies indicate a need for reclassification of the species of Diplodia listed in Saccardo (7), since it may be found that many others having similar morphological characters will have the same host range.

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DECAY OF WOOD AND GROWTH OF SOME HYMENOMYCETES AS AFFECTED BY TEMPERATURE[1]

RALPH M. LINDGREN

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Investigations pertaining to the effect of temperature on the development of wood-destroying fungi have been limited largely to determining the cardinal points for spore germination and for mycelial growth on culture media and to studying the effect of heat in arresting their development in wood. So far as known, there have been no attempts to determine by means of laboratory experiments the relation of temperature to the rate of wood decay. The opportunity to study the relation of temperature to decay of wood and mycelial growth on culture media was supplied in the course of investigations of the effect of some fungi on the mechanical properties of wood. Although the work attempted was not comprehensive, the results may be of interest and are recorded briefly in this paper.

MATERIALS AND METHODS

The fungi used were *Lenzites sepiaria* Fries, *Polystictus versicolor* (L.) Fries, and *Lentinus tigrinus* Fries. The original cultures, with the exception of *L. tigrinus,* were obtained from the Bureau of Forest Pathology, Forest Products Laboratory, Madison, Wisconsin. *Lenzites sepiaria* and *P. versicolor* are wood-destroying fungi of great commercial importance on forest products, the former occurring on softwood and the latter on hardwood timber, principally. *Lentinus tigrinus* is typically a hardwood-rotting organism but is of little economic significance.

Ninety-millimeter Petri dishes, containing 10 cc. of malt-extract agar, were used in studying the effect of temperature on mycelial growth on culture media. The medium was prepared as follows: Trommers malt extract (plain), 25 gm.; agar agar (Bacto granulated), 20 gm.; distilled water, 1,000 cc. Uniform squares of mycelium taken from the growing edge of a plate culture of each fungus were placed in the center of the Petri dishes. These plates were distributed immediately to 13 incubators, which ranged in temperature from 0 to 50° C. Three different series were used, each one consisting of 5 plates for each organism at each temperature. Two diameter measurements in millimeters were taken of each mycelial disk at 24-hour intervals for a period of 8 days. The average of the 2 measurements was used to represent the growth of the culture at the time of measurement.

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The average growth in diameter at the end of any given period was based on the average of the 15 measurements taken for each organism at each temperature.

In studying the effect of temperature on the rate of wood decay, blocks of *Pinus resinosa* Solander, *Picea glauca* (Moench) Voss, *Betula papyrifera* Marshall, and *Populus tremuloides* Michaux were rotted under controlled temperature conditions. The blocks, 112 in number, and $1 \times 1 \times 3$ in., were oven-dried at 100° C. to a constant weight, weighed, and soaked in water until a moisture content of approximately 100 per cent, oven-dry basis, was reached. They were then autoclaved for 30 minutes at 15-pounds pressure and a block of each species of wood was transferred to 2-qt. fruit jars in which pure cultures of *Lenzites sepiaria, Polystictus versicolor,* and *Lentinus tigrinus* had become established on agar. The culture jars, 28 in number, were placed immediately in incubators maintained at constant temperatures of 22, 27, 32, and 35° C. After an incubation period of 2

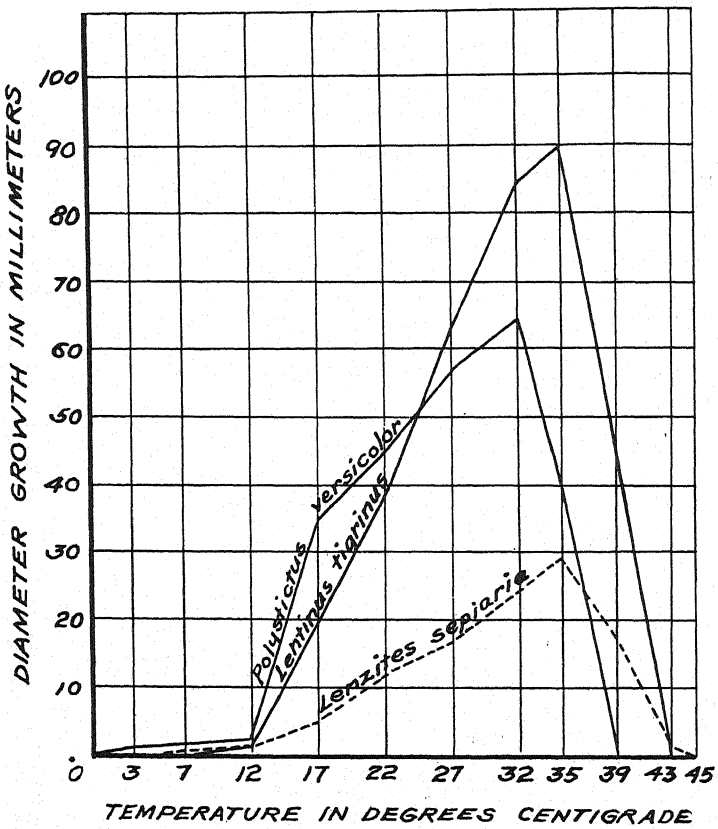


FIG. 1. Four days' mycelial growth of *Lenzites sepiaria, Lentinus tigrinus,* and *Polystictus versicolor* on malt-extract agar under different temperature conditions.

months, the decaying blocks were removed from the jars and their oven-dry weights again recorded. The relative losses in weight induced by decay at the different temperatures were determined from the original and final oven-dry weights.

RESULTS

Mycelial Growth on Culture Media

The mean growth in diameter in millimeters of the several fungi after various periods of exposure and for 4 days' growth at different temperatures is shown in table 1 and figure 1, respectively. It is noted that the mean

TABLE 1.—*The mean diameter growth on malt-extract agar of Lenzites sepiaria, Lentinus tigrinus, and Polystictus versicolor after various periods of exposure at different temperatures*

Organism	Days	Average diameter growth in millimeters at various temperatures in °C.												
		50	45	43	39	35	32	27	22	17	12	7	3	0
Lenzites sepiaria	1	0	0	0	+[a]	3	2	+	+	+	0	0	0	0
	2	0	0	0	2	10	7	2	+	+	0	0	0	0
	3	0	0	+	7	21	17	12	7	3	0	0	0	0
	4	0	0	+	17	29	25	17	12	5	+	0	0	0
	5	0	+	1	23	40	38	25	19	12	+	+	0	0
	6	0	+	2	28	55	52	38	27	17	1	+	0	0
	7	0	+	3	35	65	61	47	33	22	3	+	0	0
	8	0	+	4	44	77	73	57	40	28	8	2	0	0
	15	0	+	14	–	–	–	–	–	70	21	10	0	0
Lentinus tigrinus	1	0	0	0	+	12	10	7	2	+	0	0	0	0
	2	0	0	0	7	39	34	20	9	2	+	0	0	0
	3	0	0	0	25	75	65	41	21	7	+	0	0	0
	4	0	0	0	44	90	85	63	38	19	+	+	0	0
	5	0	0	0	58	–	–	80	54	26	2	+	0	0
	6	0	0	0	73	–	–	–	68	35	7	+	0	0
	7	0	0	0	90	–	–	–	80	46	10	+	0	0
	8	0	0	0	–	–	–	–	90	55	12	+	0	0
	15	0	0	0	–	–	–	–	–	–	40	2	0	0
Polystictus versicolor	1	0	0	0	0	9	12	11	7	2	0	0	0	0
	2	0	0	0	+	18	28	25	18	9	+	0	0	0
	3	0	0	0	+	32	47	42	32	19	+	+	+	0
	4	0	0	0	+	40	65	57	45	35	2	1	+	+
	5	0	0	0	+	50	83	73	56	47	7	2	1	+
	6	0	0	0	1	56	90+	90	69	59	11	3	2	+
	7	0	0	0	2	62	–	–	81	70	15	5	3	1
	8	0	0	0	2	70	–	–	90	79	19	8	5	2

[a] + indicates trace of growth.

increment at any given period of exposure increases progressively as the optimum temperature, based on the greatest mean diameter growth, is approached. The mean growth rate for the individual successive 24 hour-periods is not always the same. Although the mean diameter growth for the entire period is consistently greater at the optimum than at any other temperature, increments for given 24-hour exposure periods are sometimes greatest at temperatures other than the optimum ones. Growth rate is found to vary to some extent with time of exposure, but there is no definite progressive decrease in daily increment at higher temperatures nor progressive increase of daily increment at lower temperatures, as has been reported by some investigators.

Of the 3 fungi, *Lenzites sepiaria* showed the slowest rate of growth, 9 days being required to cover a 90-millimeter Petri dish at the optimum temperature. *Lentinus tigrinus* and *Polystictus versicolor* covered the same area in approximately 4 days and 5½ days, respectively. The cardinal temperatures for growth of *Lenzites sepiaria* were found to be as follows: optimum 32–35° C.; minimum, approximately 5° C.; and maximum, 45° C. These results vary slightly from those obtained by Snell (5) but agree rather closely with those reported by Falck (2, pp. 9–32) for this same fungus. Snell found the optimum to be between 30 and 34° C.; the minimum, approximately 8° C.; and the maximum, 40 to 44° C.; while Falck reported 35° C. as the optimum, 5° C. as the minimum, and 44° C. as the maximum point. Humphrey (4) states that the optimum for development rests between 28 and 32° C. The use of different strains of this organism could easily account for such variations in the temperature relations.

Lentinus tigrinus grew more rapidly but had a narrower range of growth than either of the other fungi. It had the same comparatively high optimum temperature for growth, 32 to 35° C., as *Lenzites sepiaria,* but its extreme limits were not so widely divergent, the minimum being approximately 7° C. and the maximum 40 to 43° C. An increase of a few degrees above the optimum temperature retarded growth to a much greater extent than a corresponding decrease below it. This is strikingly evident from a comparison of the growth figures at 22, 32, and 43° C. At the end of 4 days, growth at 22° C. was almost ½ that at 32° C., while, at 43° C., growth had been inhibited.

The cardinal temperatures of *Polystictus versicolor* were the lowest of all, its optimum point being between 27 and 32° C., the minimum at approximately 0° C., and the maximum about 40° C. The retardation of growth resulting from a slight increase in temperature above the optimum was decidedly pronounced. It is noted from table 1 that an increase of 9° above was practically equivalent to a decrease of 23° below the optimum in

its retarding influence on growth. An increase of less than 10° C. inhibited growth, while a decrease of 30° C. was needed to accomplish the same result.

Decay of Wood Induced at Different Temperatures

The decay of wood induced by the 3 organisms under different temperature conditions, as determined from the relative losses in weight of wood, is shown in table 2 and figure 2. For the purposes of this discussion, the

TABLE 2.—*The average percentage loss in weight of wood rotted under different temperatures for a 2-month period*

Organism	Temperature in °C.	Average percentage loss in weight of wood				Average loss in weight for all woods
		Pine	Spruce	Birch	Aspen	
Lenzites sepiaria	35	5.20	5.20	3.50	6.00	4.97
	32	6.60	3.70	3.10	3.20	4.15
	27	3.50	3.90	3.00	3.30	3.43
	22	3.40	3.10	2.20	3.70	3.10
Lentinus tigrinus	35	0.80	2.20	7.10	7.00	4.28
	32	1.60	3.80	5.20	6.80	4.35
	27	3.50	2.00	9.00	10.00	6.12
	22	1.90	0.90	3.40	4.80	2.75
Polystictus versicolor	35	2.00	1.40	7.40	4.80	3.90
	32	2.30	3.60	11.40	8.20	6.38
	27	3.70	3.00	6.70	5.50	4.73
	22	1.30	2.00	3.90	6.00	3.30

aggregate or average loss in weight for all woods will be used as the basis for comparing the rates of decay at different temperatures. It will be seen in table 2, however, that the decay-temperature relationship for any one organism is not consistent for all the woods.

A comparison of figures 1 and 2 indicates a mycelial-growth-temperature and decay-temperature relationship in the case of *Lenzites sepiaria* and *Polystictus versicolor*. Decay caused by these 2 organisms progressed most rapidly at temperatures that were the optimum ones for their mycelial growth on culture media. Thus, the loss in weight produced by *L. sepiaria* was greatest at 35° C. and was successively reduced at each drop in temperature below this point. At 22° C. the average loss in weight due to decay was only 63 per cent of that induced at 35° C. Decay caused by *P. versicolor* progressed at a maximum rate at 32° C. but was fairly rapid at 27° C., also. At 35 and 22° C., the losses in weight were only 61 and 53 per cent of those at 32° C. It is noted in figure 1 that the mycelial growth of this organism was also greatest at 32 and 27° C. and least at 22° C.

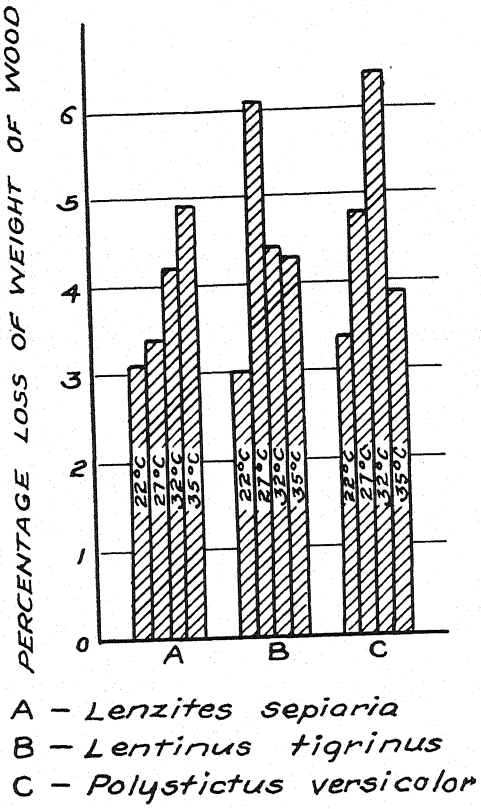


FIG. 2. The aggregate loss in weight of wood induced by *Lenzites sepiaria, Lentinus tigrinus,* and *Polystictus versicolor* under different temperature conditions.

A relationship between rate of decay and mycelial growth at different temperatures was not indicated in the case of *Lentinus tigrinus.* Decay induced by this organism progressed most rapidly at 27° C., while growth of mycelium on culture media was greatest at 35 and 32° C. However, it is seen in table 1 that mycelial growth was fairly rapid at 27° C. as well as at the optimum temperatures. Both decay of wood and mycelial development were materially retarded at a temperature of 22° C.

The 3 fungi varied somewhat in their respective abilities to attack the several woods employed in the decay studies. *Lenzites sepiaria* caused the greatest losses in weight in the 2 coniferous woods, pine and spruce, and was active in decaying birch and aspen, also. *Polystictus versicolor* produced pronounced losses in weight in both of the hardwoods and developed fairly rapidly on the coniferous woods. *Lentinus tigrinus* was distinctly a hardwood-destroying organism, producing decay in birch and aspen equal

to that induced by *P. versicolor* but making little progress in attacking the coniferous woods. Snell (6) reported similar results with *L. tigrinus* and concluded that the rotting potentialities of this organism were limited largely to hardwood timber.

Considering aggregate loss in weight in all temperatures as the criterion of susceptibility to decay, birch proved relatively resistant to *Lenzites sepiaria* but was readily attacked by *Lentinus tigrinus* and *Polystictus versicolor*. Norway pine and white spruce were decayed rather rapidly by *Lenzites sepiaria* but proved more resistant to *Lentinus tigrinus* and *P. versicolor* than either of the hardwood species. These 2 coniferous woods differed little in their relative susceptibility to the several fungi. Aspen was quite readily subject to decay by all 3 organisms.

DISCUSSION

The temperature relations of wood-destroying fungi have been studied on culture media only, probably because this method is the simplest and gives most promise of yielding immediate and consistent results. Since no correlations have been made between the rate of decay of wood and the rate of mycelial growth on culture media at different temperatures, it has been assumed that the temperature relations of an organism on wood could be predicted from the results of tests on agar. In all probability, this assumption is sufficiently correct for all practical purposes, but there are two possible reasons for suspecting that it may not be entirely true. The first concerns the chemical and physical differences between the agar and the wood. It has been demonstrated by a number of investigators that the temperature relations of fungi may vary with the substratum upon which they are grown. Brooks and Cooley (1), for instance, in their study of stone-fruit fungi, found an interesting contrast between the behavior of *Sclerotinia cinerea* (Bon) Wor. and *Rhizopus nigricans* Ehr. when grown at different temperatures on peaches and on potato-dextrose agar, the former organism growing earlier and more rapidly at the lower temperatures on peaches than on agar, while the reverse condition held for Rhizopus. It is conceivable that differences in wood and agar as substrata alter the temperature relations of wood-destroying fungi as well.

The second possible difficulty in predicting the rate of wood decay at various temperatures from tests on agar lies in the time factor involved, agar tests covering relatively short experimentation periods, while decay of wood requires long exposure periods that may continue for years. Fawcett (3) has emphasized the need of considering the influence of time on rate of growth and has shown for certain fungi that growth rates vary for different consecutive observation periods at the same maintained temperature. With

culture periods of from 3 to 6 days and an observation period of 24 hours, it was found for the fungi studied that, in general, the optimum temperature for growth shifted to lower temperatures with each successive observation period and that there was a corresponding displacement of the apparent maximum point. With comparatively low temperatures, the growth rate increased with the age of the culture, and with the highest temperatures it decreased throughout the culture period. If such changes in the growth-temperature relations with different periods of exposure hold true for wood-destroying fungi, tests on agar would indicate the optimum conditions, and possibly the maximum and minimum points, for the initial part of the period of growth in wood only, and a decidedly lower optimum might be expected for decay, continuing through several months and even years. In the present study on agar, there is noted a slight tendency for the growth rate at the higher temperatures to be retarded towards the end of the experimental period, as might be expected from Fawcett's results, but the data are too inconsistent to permit any conclusions. Alhough the results on agar do not indicate definitely that long exposure periods lower the optimum temperature for growth of any of the organisms, it is possible that such a time factor contributed towards the divergence between the results on agar and on wood in the case of *Lentinus tigrinus*.

The work reported in the present paper justifies few definite conclusions because of its preliminary nature and certain irregularities presented in its results. It indicates for the fungi studied that temperatures favorable for mycelial growth are, likewise, conducive to rapid decay, but whether the limits or relative rates of growth are the same on agar and in wood has not been determined. It is hoped that more intensive investigations of these phases of the life history of the more common wood-destroying fungi, possibly using relative losses in weight or the progressive evolution of carbon dioxide as an index of decay, will be stimulated as a result of this report. Such studies will not only contribute to our knowledge of the biology of these organisms but will also have a practical application in the formulation of control practices.

SUMMARY

The effect of temperature on the rate of wood decay and of mycelial growth on culture media was studied under controlled conditions for 3 wood-destroying fungi, *Lenzites sepiaria, Polystictus versicolor,* and *Lentinus tigrinus*. The cardinal temperatures for mycelial growth on malt-extract agar were as follows: *Lenzites sepiaria,* optimum 32–35° C., minimum approximately 5° C., and maximum 45° C.; *Lentinus tigrinus,* optimum 32–35° C., minimum approximately 7° C., and maximum 43° C.;

Polystictus versicolor, optimum 27–32° C., minimum approximately 0° C., and maximum 40° C.

Decay caused by *Lenzites sepiaria* and *Polystictus versicolor* progressed most rapidly at those temperatures most favorable for their mycelial growth on culture media. Decay induced by *Lentinus tigrinus* was most rapid at 27° C., while greatest mycelial development occurred at 35 and 32° C. At a temperature of 22° C., decay processes as well as growth of mycelium were materially retarded for all 3 organisms.

Attention is called to the need for correlating tests on agar with decay studies on wood where the temperature relations of wood-destroying fungi are being determined. Mention is made of the differences both in the chemical and physical nature of the substrata and in the duration of fungous development as reasons why predictions of the rate of wood decay at different temperatures on the basis of tests on agar are not reliable.

DIVISION OF PLANT PATHOLOGY AND BOTANY,
UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

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TRANSMISSIBILITY OF THE BROOMING DISEASE OF BLACK LOCUST

L. W. R. Jackson and Carl Hartley

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In 1898 a witches' broom on black locust, *Robinia pseudacacia* L. was recorded in a brief note by Waters (12). Overlooking this report, Hartley and Haasis (4) recently published a description of a malady that they designated "brooming disease." The two diseases are almost certainly identical. The experimental data in the present paper, though not extensive, combine with field observations to indicate strongly that this locust disease is of the peach-yellows type and is presumably caused by a virus. Particular interest attaches to this disease, partly because it adds to the brief list of viroses of woody plants, which, because of the nature of the hosts, are particularly good material on which to study recovery from virus infections, and partly because it is, to date, the only virus disease that has been described from the rich American forest-tree flora, which includes hundreds of timber-producing species. The parasitic sandalwood, *Santalum album* L., of Asia, is the only other timber tree for which a published report of a virus disease has been noted. Our wild plum, host for peach rosette (11), is scarcely a forest tree in the usual sense of the word.

Histological and cultural studies made thus far have failed to reveal a visible causal organism. The histological technique employed by Heald and Sprague (5) in identifying the fungus associated with the witches' broom of service berry failed to show any trace of a fungus in either the periderm or cortex of the proliferated twigs of black locust. A fungus growing superficially on the bark and tentatively referred to Camarosporium will be described more fully in a later paper. It should be mentioned that Waters reported the same experience with the witches' broom on black locust collected in 1898. J. E. Humphrey thoroughly studied his specimens "and to his surprise found not the slightest trace of fungus hyphae or fructification in or on the tissues. Under the microscope, thin sections showed that the wood was very much gnarled and twisted, and a section through a node resembled somewhat a piece of bird's eye maple. We must, therefore, ascribe the formation of so many adventitious buds and such abnormal foliage to something else than fungus enemies."

Since the brooming disease could not be attributed to the presence of a visible pathogen, the next logical step was to determine the transmissibility of the disease.

TABLE 1.—*Experiments on transmission; grafts made March 16, 1929*

Scions	Source of scions: Branch number	Source of scions: Portion of branch	Graft method	Graft serial number	Scion growth (1929): April 19	Scion growth (1929): May 14	Scion growth (1929): September	Brooming September, 1929
	1	Terminal	Cleft	1	Slow	Vigorous	Alive	9 in. below graft
	2	Basal	"	2	Fair	"	"	None
	3	Terminal	"	3	Good	"	"	"
	4	"	"	4	1 bud	Poor	Dead	"
	5	Basal	"	5	Good	Good	Tag lost	"
	6	"	"	6	1 bud	Slow	" "	"
Broomed	7	Terminal	"	7	Good	Good	Alive	14 in. below graft
	8	"	Veneer	10	None	None	Dead	None
	9	"	"	12	2 leaves	1 leaf	Good	Slight
	10	"	"	14	Sparse	Sparse	Alive	28 in. below graft (Fig. 1)
	11	"	"	16	None	None	Dead	On lateral branch (Fig. 2)
	12	"	"	19	"	"	Good	None
	7	Basal	Cleft	8	Excellent	Good	Alive	"
Apparently	8	"	"	9	None	"	Dead	"
normal	9	"	"	11	Fair	"	Tag lost	"
but	10	"	"	15	None	"	Alive	Mild, to 1 ft. below graft
from	11	"	"	17	"	None	Dead	None
broomed	12	"	"	18	1 bud	"	"	"
branch[a]	13	"	"	20	1 bud	Good	Good	"
From normal	14	"	"	21	Good	"	"	"
branch in	15	"	"	22	Fair	"	"	"
broomed	16	"	"	23	Good	"	"	"
sprout cluster	17	——	"	24	None	None	Dying	"
From	18	——	"	25	Good	Good	Good	"
normal	19	——	"	26	"	"	"	"
sprout	20	——	"	27	"	"	"	"
cluster								

[a] Taken from 12 in. below lowest broom.

GRAFTING EXPERIMENTS

Normal and diseased scions were top-worked with cleft or veneer grafts on healthy seedling stocks. In the cleft grafts each stock bore 2 scions. The seedling stocks were about 4 years old and had an average height of 3 ft. and diameter less than 1 in. at the time of the grafting. The scions were gathered from several widely separated points in the District of Columbia. The normal scions or checks were cut from sprout clusters that bore no visible evidence of disease history. Each diseased scion was cut from a densely proliferated broom and an apparently normal scion was cut at a distance of approximately 12 in. inwardly on the branch from the last point of visible brooming. The purpose of this second set of normal scions, which was cut from diseased branches, was to determine the systemic distribution of the infective principle. All the scions were dormant and the grafting was done March 16, 1929. Details as to the source and type of scions and their growth are given in table 1.

Of a total of 12 grafts with diseased scions, the scions grew through the summer on at least 7, and 4 of these, together with 1 of those on which the scion had promptly died, showed definite brooming of the stock before the end of the first summer. (See Figs. 1 and 2.) Of a total of 6 grafts with apparently healthy scions from branches that showed diseased terminals, scions were growing 2 months later on 4, and 1 of these developed brooming of the stock. The stocks with lost tags are included among those with no brooming, since, in September, none of the unmarked trees in the planting showed brooms. All of the broomed trees, except that shown in figure 2, were dead the following spring. In each of the 6 cases of positive transmission the brooming symptoms included twig proliferation and leaf dwarfing that are identical with the symptoms observed in the field. Details are presented in table 1.

None of the grafts with scions from normal branches and none of the 39 nongrafted seedlings in the same planting, some in physical contact with the broomed trees, showed any visible brooming symptoms, either in 1929 or the 2 subsequent years. Therefore, it does not appear that the disease is strongly contagious and likely to spread through mere contact or proximity of normal and infected plants.

The apparent lack of need for positive union of the graft and subsequent growth in order to secure transmission, indicated by the single case of Graft 16 (Fig. 2), suggests a dissimilarity with other tree viruses. Graft 16 formed no union and showed no signs of growth, yet the stock developed a definite broom on one of its branches. It has been repeatedly demonstrated that the successful transmission of peach yellows is dependent on a positive union of the grafts (8). In the case of phony disease of peach

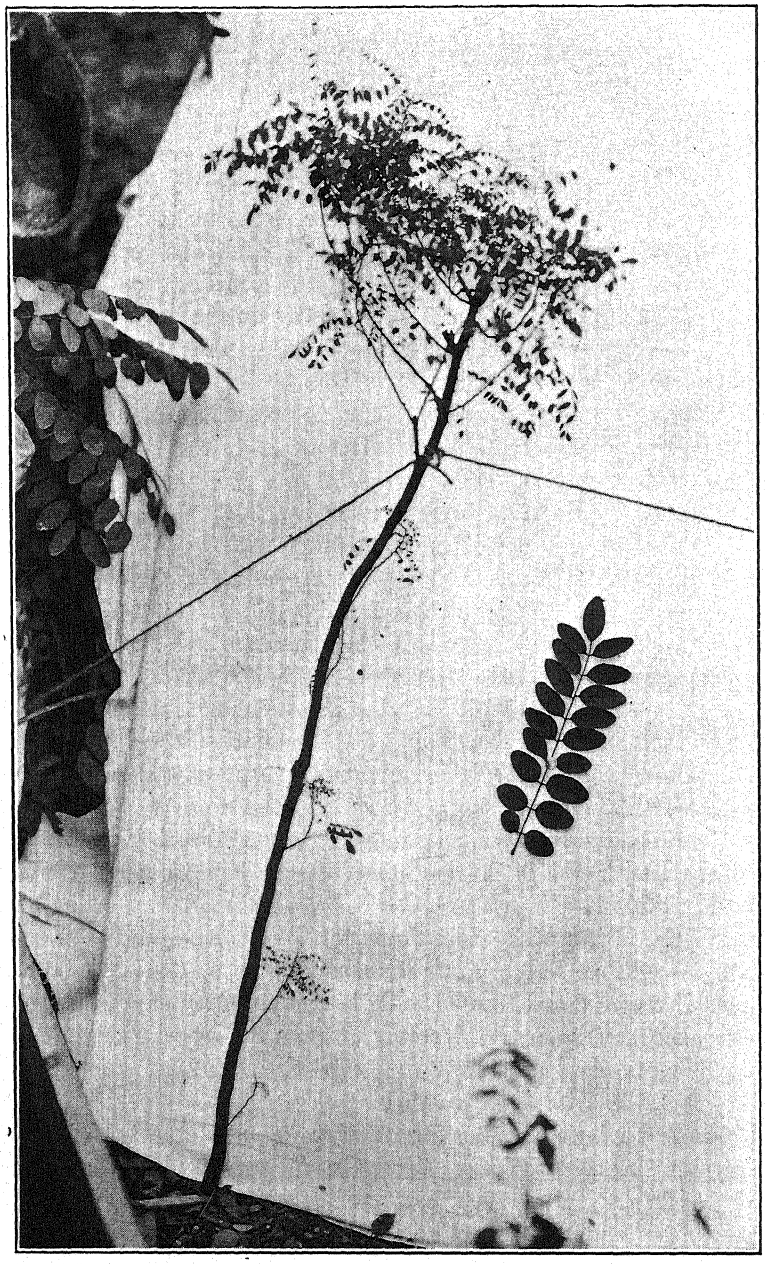


FIG. 1. Graft 14, showing extent of brooming of the stock 5 months after grafting, as compared with a normal black-locust leaf. Cleft graft at top of trunk.



FIG. 2. Graft 16, showing single terminal branch broom. A veneer graft was made at x, which never formed a union.

(6) normal nursery trees contract the disease when root grafts form a positive union but fail when grafts are negative. Juice injections have failed to transmit such virus diseases as peach yellows, little peach, and spike of sandalwood (9). Nevertheless, in view of the fact that a single scion in these black-locust grafts apparently transmitted the disease without union, it seems logical to attempt juice-injection experiments.

The length of time after grafting required for the appearance on the stock of the symptoms of the disease is in agreement with many virus diseases already reported. In this experiment the black-locust stocks bore well-developed brooms during the same growing season, 5 months after grafting. The grafts were made March 16, 1929, and brooms on the stocks were fully developed by September. This is a short period when compared with that for peach yellows, little peach (Waite, oral communication), and phony disease (6), in which the symptoms do not appear on the stocks till the following season; but longer than for peach rosette (8) and spike of sandalwood (3), in which visible evidence of transmission has been obtained in 2 months. Four months is given as the time required for evidence of transmission through grafts of "buckskin" disease of cherry (7). In the case of witches' broom of ocean spray (*Holodiscus discolor*) (13) the transmitted symptoms are confined to the region of the graft the first season and do not appear in other parts of the plant until the second season. It appears that the length of period from grafting to the development of definite symptoms of the disease on the stock is an important differentiating characteristic of these supposedly virus diseases.

The amount of brooming of the stocks varied from a single terminal broom on a branch (Fig. 2) to complete brooming (Fig. 1). Graft 16 was a particularly interesting case. This graft was previously referred to as one where transmission occurred without apparent union of the graft. A veneer graft was made on the main stem, shown at point (x) in figure 2. Not only did the scion fail to unite, but a bark lesion developed at the wound, which was the apparent cause of the death of the leader 15 months after grafting. The broom appeared terminally on one of the lower branches (Fig. 2). In this case the infective principle had to be transmitted downward through the stem for a distance of approximately 8 in. and then outward to the tip of the branch, which was another 2 ft. This gives a total distance of transmission of approximately 3 ft. No brooming appeared anywhere else on the stock, and during the following 2 years the originally broomed branch has grown normally. For the buckskin graft-infectious disease (7) the greatest distance for transmission from diseased scion to healthy scion during the first 4 months was 2 ft.

The degree of brooming for the other grafts is given in table 1. In general, the outbreak of brooms where transmission was successful was simul-

taneous throughout the entire stock. In nature the brooms commonly occur terminally on sprouts, as shown in figure 2. The simultaneous development of brooming throughout the entire stock, as in figure 1, is another point that closely relates the symptoms of the brooming disease to spike in sandalwood. In transmitting the spike disease from one tree to another through root haustoria (3) the disease appeared simultaneously in all parts of the tree and practically every twig was spiked. The apparent recovery of the locust that showed the least initial symptoms of the disease is in accord with the experience with raspberries (1).

Another important characteristic derived from these transmission data is that the infective principle is actively present and transmissible in the stem and twigs of the black locust. Dissimilarly, the virus causing the phony disease of peach is restricted to the roots and so far it has not been transmitted by means of stem material. In the field it has been observed that locust roots frequently send up completely proliferated sprouts. These broomed sprouts seldom attain a height greater than 18 in. and usually die back at the end of the first growing season, but the same root can give rise to additional broomed sprouts in following seasons. It appears, therefore, that the infective principle is actively present in black-locust roots. So far no attempt has been made to transmit the disease by means of root grafts.

BUDDING EXPERIMENTS

An attempt was made to transmit the brooming disease of black locust by budding. A large number of diseased and normal buds were collected from various field plots in the District of Columbia in late summer, 1929, and budded on to normal seedling stock. None of the buds made union, and the following spring they were all dead. To the end of 1931 none of the budded stocks has shown any visible signs of brooming. The failure of budding may be attributable in part to the morphology of black-locust buds. As shown by Büsgen and Münch (2), the new bud is deeply embedded in the petiole pulvinus. When the petiole is cut off for budding purposes the stub dries up and perhaps hinders the growth and development of the new bud the ensuing spring. The budding method has proved unsuccessful in the experiments with spike of sandalwood (10). The "patch graft" used recently by Sreenivasaya is probably worth trying in future black-locust brooming-disease experiments.

CONCLUSIONS

The brooming disease of black locust has been successfully transmitted in one case to healthy stocks through grafted diseased-stem scions despite the death of the scion. In certain cases the disease failed of transmission despite the union and growth of the scion.

Brooming appeared on the stocks, during the first season, in some cases nearly 3 ft. from the graft. The time requirement for visible evidence of transmission was from 4 to 5 months. One of the infected trees apparently recovered.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

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KNOB AND ELEVATED VEIN FORMATIONS ON SWEET-POTATO ROOTS[1]

R. F. POOLE

(Accepted for publication April 2, 1932)

There are two specific disorders of the sweet potato that cause the edible root to vary from the normally developed smooth, symmetrical, and uniform shape, denoting quality, to that of an unsightly rough appearance signifying inferior quality. The phenomena are widely distributed in all areas where the crop is grown, but in any area the prevalence is so influenced by such factors as types of soil, amounts of nitrogenous substances in the soil, and precipitation during the growing season that their effects are of no importance in certain seasons and very injurious in others. The slowness in which southern varieties are being accepted in the larger markets is largely a result of inferior quality based mostly on irregular sizes, shapes, and otherwise unattractive appearance displayed because of these two disorders. Studies over a long period of years on the causes of and the factors applicable to inhibiting these two effects are discussed briefly in this article.

THE KNOB CONDITION

The knob disturbance (Fig. 1, A), being of wide occurrence, has made the symptoms well known. Probably every grower of the crop has observed the disorder. The knob character may, without careful examination, be confused with somewhat similar raised areas resulting from infestation by *Caconema radicicola* Cobb, the root-knot forming nema. In potatoes affected with only the knob condition there are no such internal disturbances of the inner tissues as occur after the nema infests the potato and develops in the tissues numerous light-color flask-shape female nemas, encircled by a ring of tissue that becomes oxidized brown in color. Other than displaying an irregular rough outward surface, the potatoes are decidedly normal throughout.

The knobbiness shown in figure 1, A, was obtained by growing the crop on Cecil sandy loam, which, during the drought period of 1930, became very hard. The root during growth gave every evidence of following the lines of least resistance in this soil. Irregular hardness was conspicuous in the soil areas where the roots were developing. The disturbance is always most pronounced on the distal end, the end deepest in the soil. The smaller potatoes as well as the feed roots often show the effects throughout. The

[1] Technical Paper No. 58, North Carolina Agricultural Experiment Station.

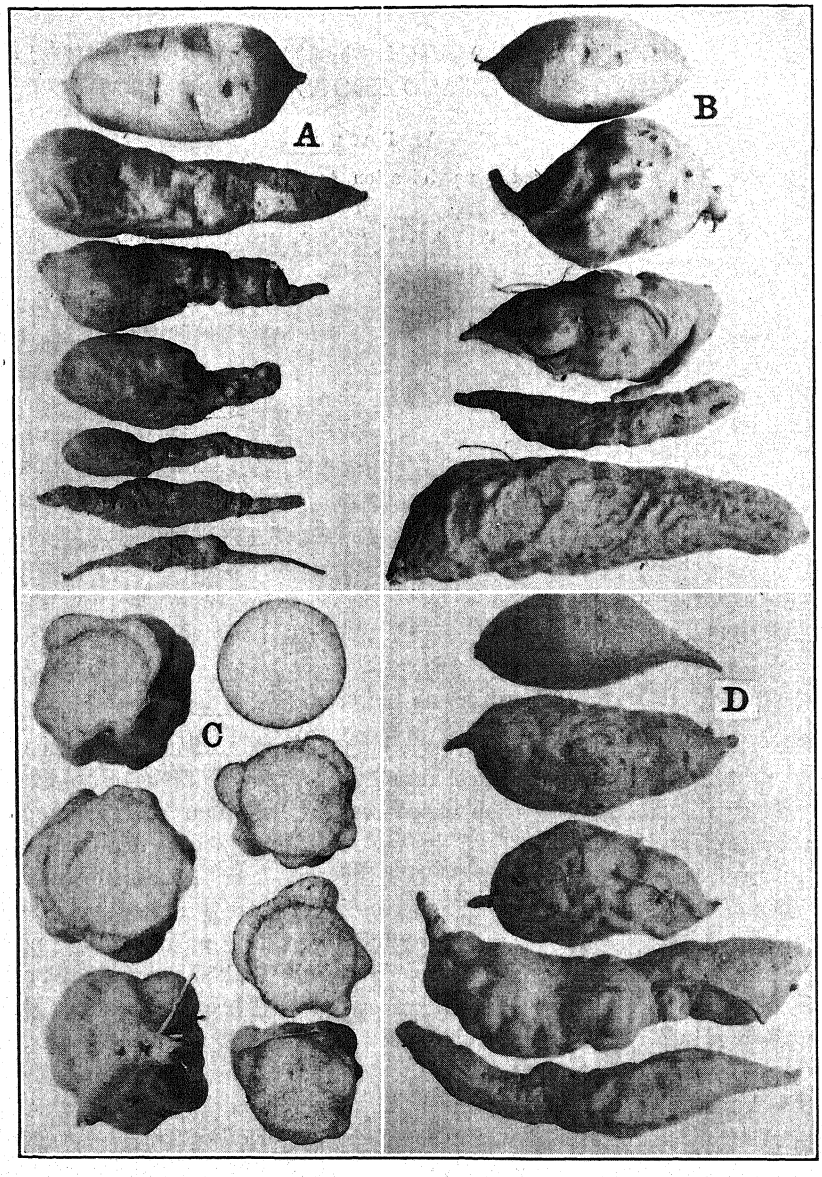


FIG. 1. A–D. Nancy Hall variety of sweet potatoes. A. Comparison of knob disorder on potatoes grown in Durham sandy loam in drought area during 1930 with a smooth symmetrically formed potato grown on loose sandy soil in the same field. B. Vein disorder of potatoes grown on deep phase Durham coarse sandy loam that was dry throughout the latter part of the summer but wet for 3 weeks previous to harvest. C. Cross sections showing the second-growth disorder in comparison with a normal potato. D. Comparison of normal potato with those showing both the knob and elevated vein condition.

crater-like cavities in the small potatoes and the deeper depressions in the larger ones were packed with hard soil when removed from the soil, indicating very great pressure of the root against the hard soil. The epidermal layer, even in the cavities, is not broken; nor is there any evidence that new periderm tissue was formed, such as develops when roots are injured by rodents. It is well demonstrated in the characteristics of the plant that root and potato development will continue regardless of the nature and character of the soil. When soils become compacted and resistant, as the clay and loamy clay soils of the Piedmont areas often do during periods of drought, the feeder roots and potato roots are forced to depart from a straight course of symmetrical development such as obtains in loose soils. When the soil is too hard to be moved readily there will be consequent knobbiness of the potatoes (Fig. 1, A). This characteristic effect is readily developed in pot cultures in clay soil under controlled conditions. During 1930, a drought season, the knob-like formation was most conspicuous on the same area and soils on which it was of no importance in the seasons of 1929 and 1931 when the soil moisture content was more favorable for plant growth and for maintaining less hardness in the soil. The injury in some instances approached complete girdling, but in no instance were any roots found completely divided. It is apparent that the sweet-potato root does not have the power to force hard soils into symmetrical molds but does have a well-developed characteristic of distributing the products in the edible roots, allowing the greatest development in the soft strata of soil. The variation in soil hardness in this area results from irregular depths of plowing soils and from turning under sandy soils when the subsoil is near the surface. Yields on the hard soils are very low, but there is no proof that other intricate factors are not of equal importance in inhibiting growth. Low soil moisture alone influences the plant to root deeply and profusely when the leaf area is large. The knob effects become very noticeable in seasons when there is an abundance of moisture during the early development of the roots, and when followed by a long period of soil-moisture deficiency, which causes the upper strata to become dry and hard. The Norfolk, Durham, and Granville sandy soils of a deep phase rarely produce the knob effect.

THE SECOND-GROWTH AND VEIN CONDITION

The potatoes shown in figures 2 and 1, B and C, have developed second growth characterized by raised vein-like markings. This condition occurs in small, large, short, and long potatoes alike. The sizes of the veins are well illustrated in figure 1, B, and in cross sections of some of the potatoes shown in figure 1, C. Some of the smaller veins are barely raised, noted only by the vein outline. Others vary in depth up to an inch in diameter.

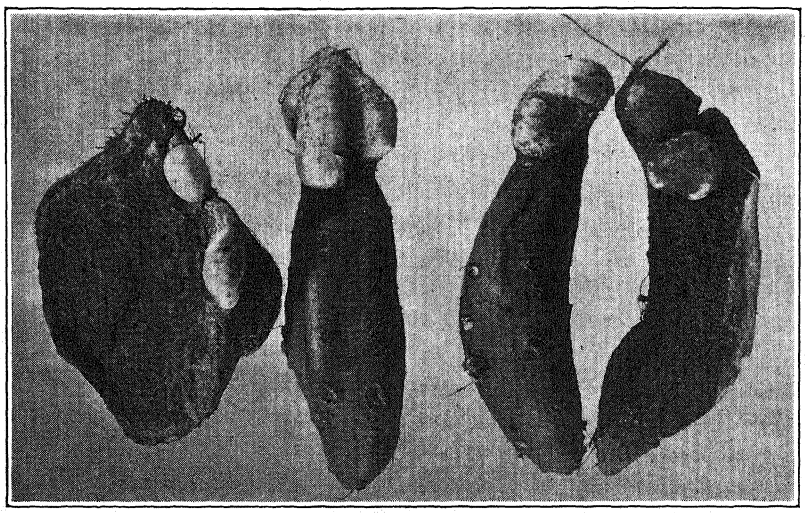


FIG. 2. Old mother potatoes producing second growth after having produced a crop of plants.

The primary cambium was permitted to oxidize sufficiently before making the photograph of figure 1, C, so as to bring out clearly and to distinguish the second-growth characters. New cambium is formed in the second growth; however, it is not clearly shown in the figure. Microscopical examination of the second-growth tissues does not indicate anything peculiarly different from that of the first growth. The edible qualities are not impaired at any time and the keeping qualities, as measured by desiccation and attacks of fungi while in storage, are just as good as those not affected by the vein condition.

A number of factors, fluctuations in soil moisture being the most important, cause second growth, but the cause of the phenomenon of vein formation is unknown. The inconsistency with which these veins occur on all sides of the potato confuses any theories set forth that veins arise from any definite sort of cells or tissues. The second-growth condition develops most prominently when low soil moisture prevails during the early development of the edible root and when this condition is followed by high soil-moisture content during the latter stages of development. The exact period of time that each condition must prevail to produce the effect is not known. Studies conducted in pots under controlled conditions have been unsatisfactory because of smallness and irregularity in shape of the potatoes.

Fertile soils, heavy nitrogenous fertilization, and such organic materials as barnyard manure and legume débris are definite aids to second growth. With sufficient soil moisture available for optimum growing conditions,

these substances force the plant to develop rank vines, on which the potatoes react suddenly to moisture conditions toward the latter part of the season.

All of the important commercial varieties are subject to second growth, but the Norton Yam and Nancy Hall varieties develop more prominently this condition than do the varieties Porto Rico and Jersey. The Big Stem Jersey strains also develop the vein more consistently than do the strains that produce smaller potatoes. The large and oldest potatoes develop the vein condition more consistently than do smaller potatoes, even on the same plant, but this does not account for the difference in susceptibility, since the variety Porto Rico, which is somewhat resistant, produces larger potatoes than does the more susceptible Nancy Hall variety. The second-growth character has been produced also on the stem of Vineless Yellow Jersey when plants are left attached to the mother potato throughout the summer in potted sand cultures (Fig. 3).

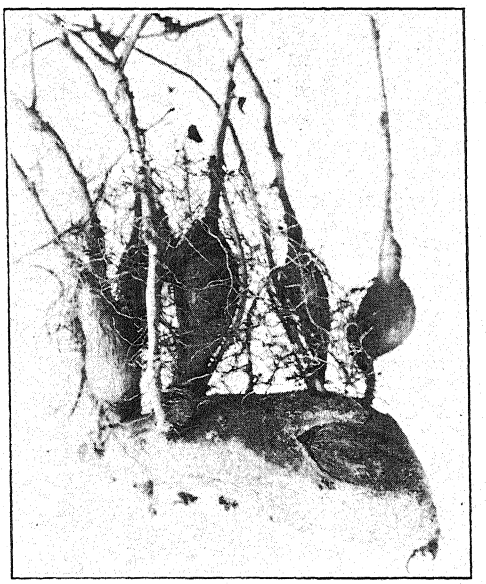


FIG. 3. Vineless Yellow Jersey potato kept in sand throughout the summer, showing plants with a well-developed second-growth condition on the stem.

DISCUSSION

The knob and elevated vein disorders may both occur on the same potatoes (Fig. 1, D). The hard soils may even influence the size of the elevated veins.

In sweet-potato culture it is well known that the development of the vines and leaf area can be advanced to such a degree that carbohydrate

development, at least that expressed by the formation of the edible root, is inhibited and delayed. The exact amount of leaf area necessary to develop the optimum quality of marketable edible roots probably varies extensively because of marked variations of growth and yields for different growing seasons. However, information on the fertilization of the crop points the way for greater uniformity of quality in the future. The use of recommended formulas of a definite analysis is open to some criticism in that there is lacking in the recommendations the necessary definite information on how and when to apply them scientifically to complex sandy soils containing a variable supply of available elements necessary for plant growth but to which the crop is most practically adapted. The sandy soils are mechanically well suited to sweet-potato development, but the character of growth and yields obtained without any other evidence shows that these soils are of the most irregular sort in maintaining the elements necessary to the growth of the plant. Even with all such factors beyond the possibility of immediate control, marked improvement in quality can be obtained and such disorders as are described here may be essentially eliminated by growing adapted varieties on well-drained sandy soils, exercizing caution with applications of nitrogenous fertilizers but with liberal applications of potassium-supplying substances.

SUMMARY

Sweet potatoes in all areas where grown are subject to 2 distinct non-pathological conditions that do not prejudice their edibility but do seriously affect their marketable quality.

The names of the conditions are referred to here as knob and vein disorders or formations.

The knob condition is easily differentiated from knots formed by *Caconema radicicola* infestation, by the absence of the oxidized brown tissues developed when caused by the latter. The knobbiness results in hard soil formed during drought periods, in which the growth of the potato is greatest in the looser soils.

The cause of the vein phenomenon is not known, but it is a result of second growth of the roots in soils of high moisture content, especially when preceded by drought conditions, and its development is aided by loamy soil types, nitrogenous fertilizers, barnyard manure, and leguminous débris.

The use of well-drained sandy soils that are adapted to the particular variety and the exercise of caution in the use of large amounts of nitrogenous fertilizers are advanced as control practices.

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION,
RALEIGH, NORTH CAROLINA.

CROWN GALL ON A CONIFER

J. G. Brown and M. M. Evans

(Accepted for publication April 19, 1932)

Cupressus arizonica Greene is a conifer native to Arizona. The tree is a valued ornamental in the Southwest because it grows as well on the mesa and in valleys as it does in a wild state on mountain slopes in its native habitat. Another quality that makes this species valuable is its resistance to Texas root rot (*Phymatotrichum omnivorum* (Shear) Duggar) which annually kills many shade and ornamental trees at the lower altitudes in southern Arizona.

A small grove of *Cupressus arizonica* (Fig. 1, F) on the campus of the University of Arizona contains individual trees that have attained a height of 40 ft. or more. The trees are growing in filled ground underlaid with caliche. Some months ago several trees in the grove showed dead tips, and, after examination by entomologists, they were declared infested with the engraver beetle, *Phloeosinus cristatus* Le Conte.[1] The infested trees were cut down and the stumps excavated. Most of the work of removing the trees was done before our attention was directed to the roots of a tree that showed numerous galls (Fig. 1, I).

The galls on the cypress superficially resembled crown galls (Fig. 1, D, C, G, H). Their surfaces were more or less rough. In size they varied from slight swellings to tumors 6 to 9 in. in diameter. One of the larger galls, but not the largest by any means, weighed 1,395 gm.

In texture most of the galls were hard, but a few spongy ones were found. The hard galls consisted of both concentric and eccentric thickenings produced by increased local activity of phellogen and cambium. Tracheids, which, of course, make up almost the entire mass of the xylem, showed transverse, oblique, and longitudinal aspects in the same cross section of a gall (Fig. 1, J). In general, the tracheids were normal in appearance. However, in some small protuberances on the surface of large galls the tracheids developed tangentially with reference to the axis of the protuberance, presented a whorled appearance, and the innermost ones were abnormally enlarged. The xylem showed growth rings, which were irregular in thickness (Fig. 1, A). In some roots sections through galls showed that the original stele at the time the gall started had partly decayed (Fig. 1, A).

[1] Vorhies, Charles T. Destroy infected cypresses. Arizona Producer (Phoenix) 9: (11): 8. Aug. 15, 1930.

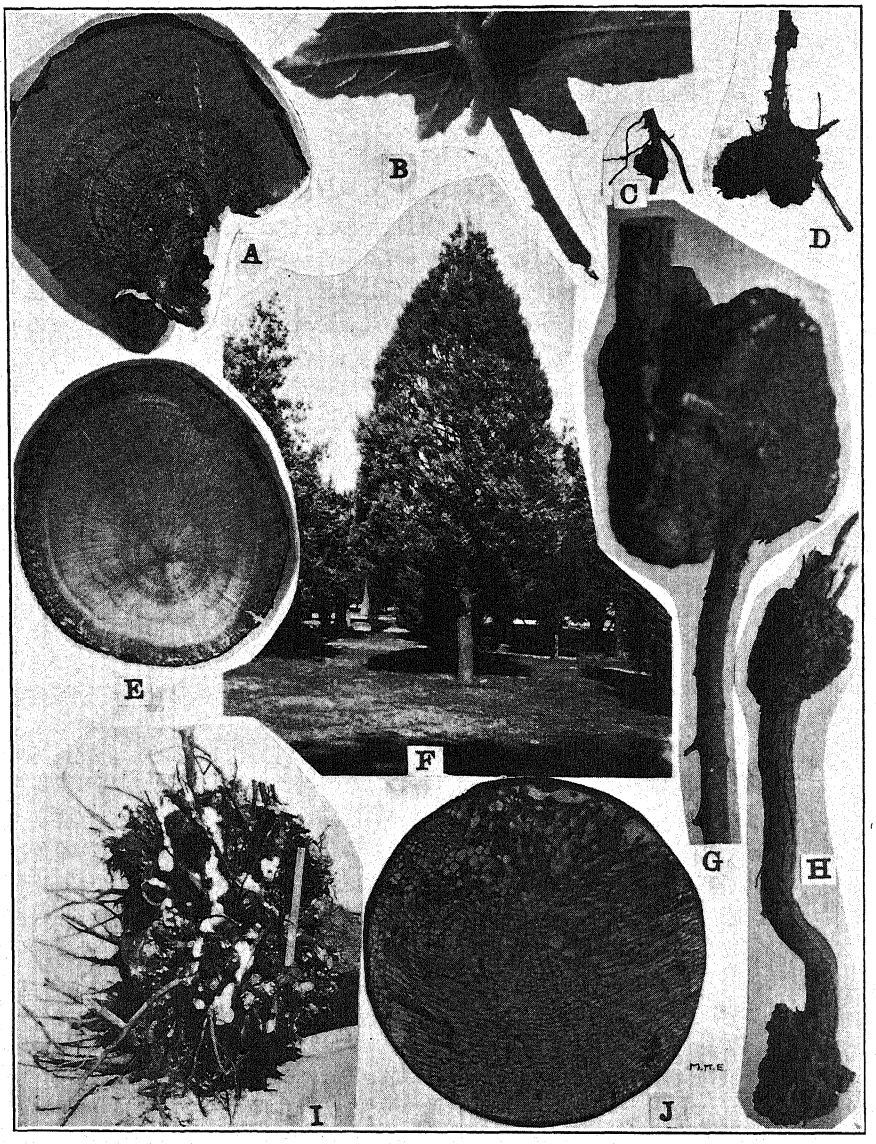


FIG. 1. Crown gall of *Cupressus arizonica*. A. Cross section of gall on partly decayed root. B. Gall resulting from needle inoculation with causal Bacterium on petiole of *Ricinus communis*. C, D, G, and H. Enlarged galls from I. E. Cross section of a healthy root. F. *Cupressus arizonica* on campus of University of Arizona. I. Crown galls outlined with cotton wool, on roots of *C. arizonica*. J. Photomicrograph of part of cross section of a gall. Note vertical, oblique, and longitudinal aspects of tracheids.

The cortex was thicker than that of normal roots and from its outer surface an abnormal sloughing-off of dead cells occurred. Living cortical cells frequently contained a resinous mass of material. The cambial zone was generally much wider in sections of the galls than in sections of normal roots, and the phloem was likewise enlarged.

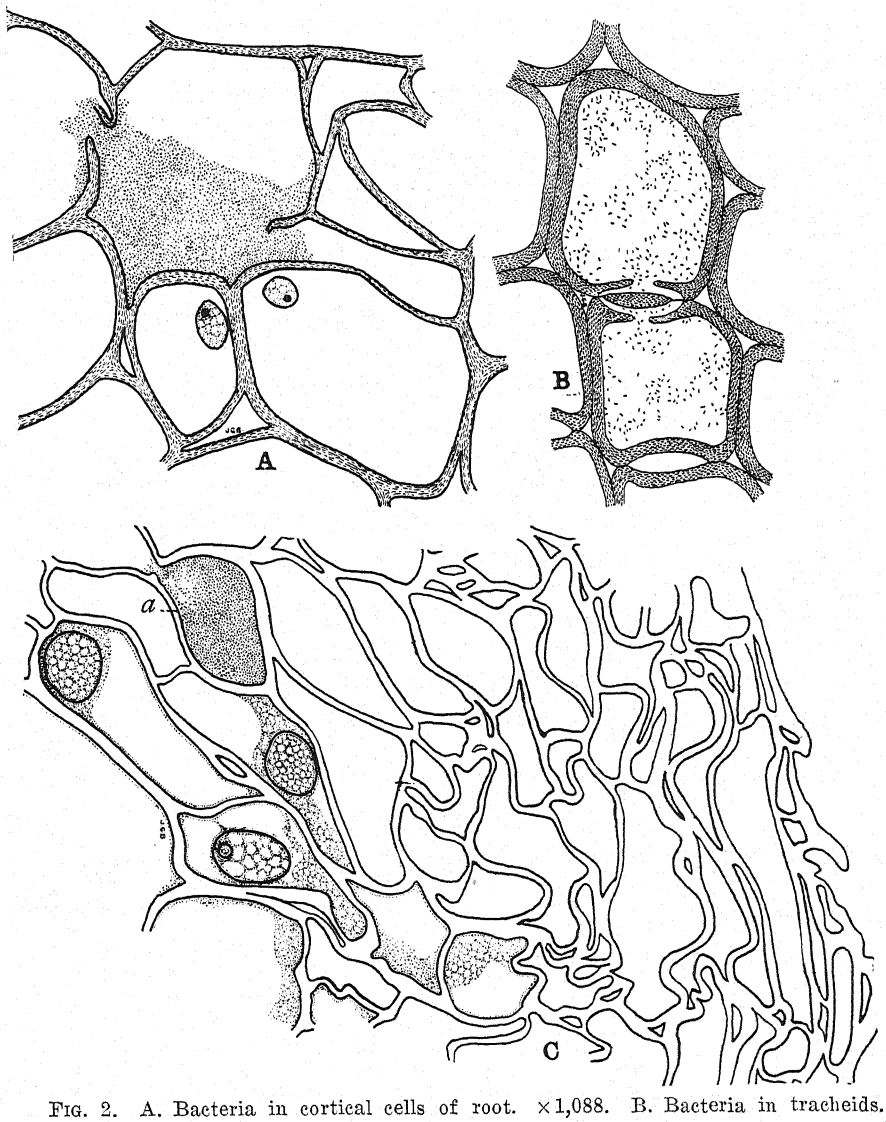


FIG. 2. A. Bacteria in cortical cells of root. ×1,088. B. Bacteria in tracheids. ×1,088. C. Part of a cross section of a gall showing cortical and subcortical cells. Bacteria in a medullary ray cell at *a*. ×554.

Living cells of the galls, as shown by stained sections, contained nuclei somewhat larger than those of normal cells in comparable tissues. No binucleate cells were found. Less starch was present in the gall cells than in similar cells of healthy roots.

Although no fungal parasites were found, either in stained microscope sections or in cultures of the gall tissues, bacteria were abundant in both. In the stained sections bacteria appeared not only in cells of the cortex but also in the cambial region and even in the tracheids (Fig. 2, B). It is probable that at least some, possibly all, of these bacterial masses represented saprophytic forms or secondary invaders. The location *inside* the cells suggests a tissue-destroying rather than a gall-producing organism.

Inoculations were made on Patel's medium[2] from gall tissues after the usual surface sterilization of the galls with 1:1000 mercuric chloride solution. From the inoculations there was isolated a motile bacterium whose physiological reactions on common laboratory media corresponded with those described for *Phytomonas tumefaciens* (Sm. and Town.) Duggar. This bacterium was inoculated into growing tissues of *Ricinus communis* L. and the inoculations were paralleled with the proper controls. It was also inoculated into many seedlings of *Cupressus arizonica*. The inoculated plants of the castor bean gave small galls (Fig. 1, B) from which the original bacterium, only, was reisolated. Even in the needle punctures in Ricinus that failed to respond to inoculation by gall production, the bacterium remained alive and was reisolated after considerable time had elapsed, in one case after 12 weeks.

Although all seedlings of *Cupressus arizonica* were grown in steam-sterilized soil, the first lot was lost through damping-off. Undue enthusiasm on the part of the investigators led to premature inoculation of the second lot of seedlings, which were killed by the needle wounds in crowns and taproots. A third lot is growing at the present time in which some seedlings are showing small swellings.

DISCUSSION

Tumors of a resinous nature on the conifers, *Pinus sylvestris* L., *P. laricio* Poir., and *P. maritima* Mill., are referred to by Dufrénoy.[3] They occurred on aerial members. Neither the tumors nor the causal organism, a coccus, agrees with the galls and the bacterium herein described.

At the time this paper on the galls of *Cupressus arizonica* was prepared for presentation at the New Orleans meeting of the American Association

[2] Patel, M. K. An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. Phytopath. **16**: 577. 1926.

[3] Dufrénoy, Jean. Sur les tumeurs bactériennes expérimentales des pins. Compt. Rend. Acad. Sci., [Paris] **169**: 545–547. 1919.

for the Advancement of Science[4] no account of crown gall on a conifer was discovered in literature by the writers. Since that time Miles and Brown[5] have reported crown gall on *Juniperus sabina* L. No inoculation studies are mentioned.

That crown gall is uncommon on the Arizona cypress seems to be true, since the senior writer has lived in Arizona for more than 22 years, has been frequently called upon to examine trees that were not doing well, and has never before observed crown gall in this species, although the disease is very common on shade trees and ornamentals. On the other hand, the Arizona cypress has been planted extensively in the cities and towns of southern Arizona only in recent years; furthermore, the occasional death of a tree, even in a country where trees are highly valued, may pass without comment or notice to plant pathologists. Again, it is possible that crown gall develops slowly on the cypress when the tree is growing in deep, fertile soil, although under similar favorable conditions for the growth of the host the disease is fatal in Arizona in a very high percentage of cases involving certain other species of trees.[6] Probably the sclerophyllous nature of the foliage of *Cupressus arizonica* and possibly also its root habit, in their relation to transpiration, favorably affect the longevity of the host.

SUMMARY

Crown gall on the roots of *Cupressus arizonica,* believed to be the first reported case of the disease on a conifer, is described with respect to the nature of the galls, the reaction of the bacterium on culture media, and the result of cross inoculations into *Ricinus communis.*

UNIVERSITY OF ARIZONA,
TUCSON, ARIZONA.

[4] Brown, J. G. Crown-gall-like hypertrophies on a conifer. (Abst.) Phytopath. 22: 4. 1932.

[5] U. S. Dept. Agr., Bur. Plant Indust. Pl. Dis. Reptr. Sup. 81; 133. 1931.

[6] Dorsey, M. J. Crown gall from the horticulturists' viewpoint. 8 pp. *In* The crown gall resolution. 41 pp. unno. Amer. Assoc. Nurserymen: Louisiana, Missouri. 1924.



PHYTOPATHOLOGICAL NOTES

The Bulbous Irises as Hosts of Tylenchus dipsaci, the Bulb or Stem Nema.—The earliest and, to our knowledge, the only literature records of bulbous iris attacked by *Tylenchus dipsaci* Kühn, are two notes by Ritzema Bos in 1925[1] and 1926,[2] both manifestly referring to the same material. Our earliest records go back to 1928, when this pest was observed almost simultaneously on iris bulbs imported from Holland and from a planting on Arlington Farm, Virginia.

A summary of our observations follows: In the period from the fall of 1928 to July, 1932, 38 lots of bulbous irises were found infested with *Tylenchus dipsaci.* Of these, 23 were foreign (Canada, England, France, and Holland) and 15 were domestic-grown (Florida, North Carolina, Oregon, South Carolina, Virginia, Washington, and Wisconsin, but mostly of foreign origin). These lots included 22 different varieties belonging to Spanish (*Iris xiphium* L.), Dutch (*I. xiphium hybridum*), English (*I. xiphioides* Ehrb.) and Morocco iris (*I. tingitana* Boiss. and Reut.).

The disease symptoms appear to be the same or very similar in all four of the species. To recognize young infections, the brown outer scales of a bulb should usually be removed. Contrary to the development in narcissus and hyacinth bulbs, where an infection usually works down into the bulb from the neck region, in bulbous irises it commonly starts from the

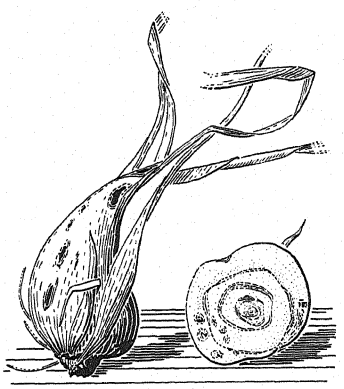


FIG. 1. Early stage of a *Tylenchus dipsaci* infestation of English iris. Exterior view shows typical spots (yellowish); cross section of the same bulb shows the infestation as small circular spots.

1 Ritzema Bos, I. *In* Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst over het Jaar 1924. Verslag. en Meded. Plantenziektenkund. Dienst Wageningen, no. 41: 16. 1925.

2 Ritzema Bos, I. Het stengelaaltje in Iris. Tijdschr. Plantenziekten. 32: 199. 1926.

side surface, where it first shows up as a yellow spot or streak. As it spreads out, its center turns brown, even grayish, changing to a lead color (Figs. 1 and 2, A). The center of the affected area may now appear faintly

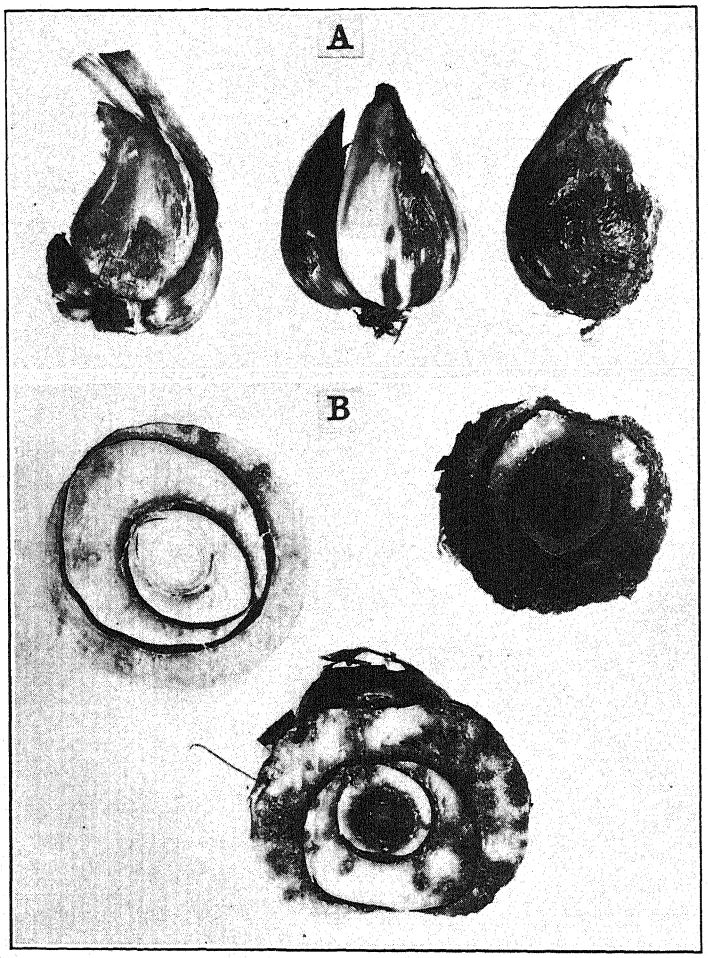


FIG. 2. A. Exterior view of various stages of *Tylenchus dipsaci* infestation of English iris bulbs. B. Cross sections of various stages. Black rings are shadows between scales and should not be confused with infected areas. Photographs through the courtesy of Dr. F. Weiss, Division of Horticultural Crops and Diseases, U. S. Department of Agriculture.

sunken, whereas in earlier stages it is sometimes slightly elevated. A cross section through the bulb at an early stage of the disease shows the infections as circular areas (Fig. 1).

If more than one original infection spot or streak is present, they may flow together as the disease progresses. Gradually the whole scale turns spongy, and is then often quite distinctly set off from the rest of the bulb. In still more advanced cases the whole bulb may be affected; the lead-color areas flow together, and small tunnels or canals may be seen with the aid of a hand lens (Fig. 2, B). Soon, decay will set in.

As far as is known, these symptoms are restricted to the bulb proper. The green parts of an infested plant exhibit no "spikkles," thus differing from the narcissus. The leaves remain backward in growth and sometimes become yellowish. Specimens of *Tylenchus dipsaci* rarely are found in the leaves. No flowers develop except when the disease is still in its earliest stages. If the pest is not checked, 5 or 6 months' time seems to suffice to kill a bulb.

Experiments attempting the transfer of *Tylenchus dipsaci* from bulbous irises (on a small scale and under greenhouse conditions) to narcissus and onions were not successful.

As a control measure a hot-water treatment, similar to that for narcissus bulbs, was tried, but no conclusive results have yet been reached. It is probable that the infestation may be controlled by a hot-water treatment, but its effect upon the iris plant itself has not been satisfactory.

It may be mentioned here that, recently, *Aphelenchoides fragariae* (Ritzema Bos) (= *Aphelenchus subtenuis* (Cobb)) has also been observed attacking iris bulbs. The symptoms seem to be the same as those described above for *Tylenchus dipsaci*.—G. STEINER and EDNA M. BUHRER, Division of Nematology, U. S. Department of Agriculture, Washington, D. C.

Field Inoculations of Pinus strobus with Sporidia of Cronartium ribicola in Minnesota.[1]—In the summer of 1927 a number of field inoculations were made with sporidia of *Cronartium ribicola* Fischer on 3 to 7-year-old *Pinus strobus* L. trees growing naturally in 2 different localities in Minnesota. The inoculating material consisted of telium-bearing leaves obtained from 2 host species, *Ribes nigrum* L. and *R. cynosbati* (L.) Mill. The purpose of this work was to determine whether pine could be inoculated successfully in the field. This paper reports the successful production of infection in pine, growing naturally, and suggests a possible difference in virulence of the sporidia from the 2 Ribes host plants.

The methods used were essentially the same as those reported by Snell and Gravatt.[2] Telium-bearing leaves of approximately the same age were

[1] Paper No. 1086 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Snell, W. H., and Annie Rathbun-Gravatt. Inoculation of *Pinus strobus* trees with sporidia of *Cronartium ribicola*. Phytopath. 15: 584–590. 1925.

carefully selected from bushes of *Ribes nigrum* and *R. cynosbati* growing in Duluth, Minnesota, and were kept in an ice box for 24 hours before use. The leaves were cut into small pieces and placed in Petri-dish moist chambers for a period of 8 hours to induce germination of the teliospores. An examination of material from both Ribes species showed abundant teliospore germination within that period. Pines, ranging in age from 3 to 7 years and growing under natural conditions in areas near Afton and Rush Lake, Minnesota, from which the Ribes had been eradicated a number of years before, were selected for inoculation. Although native Ribes had returned to these areas to some extent, no natural infection was found on either host in the region of the inoculation studies. The plants were thoroughly sprayed with water before and after the inoculum was applied. Approximately the same number of branches were inoculated at the 2 areas, inoculation being accomplished by fastening pieces of telium-bearing Ribes leaves to clusters of pine needles by wire hairpins. No effort was made to insure equal chances of infection of the pine on the 2 areas through using the same quantities of sporidia as inoculum, other than that of applying approximately the same number of leaf pieces of uniform size to each plant. The modified "iceless-refrigerator" method adopted by Snell and Gravatt for use in covering the plants was not employed in this study. Instead, wet sphagnum moss was heaped around the base of each pine and the entire plant covered by a large can, 18 in. high, over and around which wet burlap sacks and moss were placed. This type of refrigeration proved very effective in maintaining the necessary high humidity and relatively low constant temperatures around the plant during the incubation period. Droplets of water still remained on the needles 48 hours after inoculation, when the cans were removed. Maximum and minimum thermometers kept inside the moist chambers during incubation showed temperatures of 76 and 57° F., respectively.

The first examination of the pines was not made until November, 1929, 2 years and 3 months after inoculation. The results of this inspection are given in table 1.

TABLE 1.—*Results of inoculating small Pinus strobus trees in situ with sporidia of Cronartium ribicola*

Test location	Age of trees	Source of inoculum	Inoculated trees			Uninoculated trees	
			Number	Number infected	Branches infected	Number	Number infected
Rush Lake, Minn.	3–7 yr.	*Ribes nigrum*	9	8	23	2	0
Afton, Minn.	5–7 yr.	*Ribes cynosbati*	9	1	1	2	0

All trees classified as infected had one or several of the definite external symptoms of infection, such as swelling and discoloring of the stem, killing of the twigs and branches, and, in some cases, perceptible stunting of growth. The unavoidably long interval between inoculation of the pine and first examination precluded any determination of whether or not such early evidences of infection as yellow spots in the needles appeared. No pycnia or aecia had been produced on any of the plants. York *et al.*[3] found in their field studies that only a small percentage of the infections had produced pycnia or aecia 3 years after inoculation.

Of the 9 trees inoculated with sporidia from *Ribes nigrum* at Rush Lake, Minnesota, 8, or 89 per cent, of them showed definite symptoms of infection. On 2 of the diseased plants only single branch infections occurred, but in the others cankers had developed on from 3 to all of the branches of the tree. At Afton, where the source of inoculum was *R. cynosbati* instead of *R. nigrum*, only 1 of the 9 inoculated trees became infected. All non-inoculated plants, which had been kept under moist chambers and were intended to serve as controls, remained free from infection.

In examining the results of these field inoculations, the question arises as to an explanation for the pronounced difference in the amount of infection obtained in the 2 localities. Conditions subsequent to inoculations were more favorable for infection of the pines at Rush Lake than at Afton. Since little effort was made to insure equal chances of infection by using the same quantities of sporidia, there existed a possible difference in the amount of inoculum provided. Although either or both of these variables, weather and the amount of inoculum, might explain the difference in results, it seems reasonable to suggest that differences in the virulence of the sporidia of the 2 Ribes hosts might have been involved. York *et al.* report some evidence of a relation between the source of inoculum and the amount of infection produced but are doubtful whether such results indicate a difference in virulence or merely in number of the sporidia. They suggest that, if the amount of inoculum on the different Ribes species used in their study was the same, bushes of all species would be equally dangerous to near-by pines. While the differences here reported might be ascribed wholly to some similar cause, it appears that the problem of possible variations in the potency of sporidia from different sources would be worthy of careful investigation.—RALPH M. LINDGREN and A. DALE CHAPMAN, University Farm, St. Paul, Minnesota.

[3] York, H. H., W. H. Snell, and Annie Rathbun-Gravatt. The results of inoculating *Pinus strobus* with the sporidia of *Cronartium ribicola*. Jour. Agr. Res. 34: 497–510. 1927.

A Disease of Colorado Fir.—In the spring of 1931, a disease attacking the new growth of a number of Colorado firs, *Abies concolor,* was conspicuous in a planting of about 500 trees in eastern Massachusetts. From a distance the trees appeared as though injured by late frosts. The needles of the new growth were affected before they reached maturity. They became reddish and shriveled, then dark brown or black. A considerable number of the dried needles clung to the twigs for some time, but by midsummer a second growth of stunted or malformed needles had taken the place of some of the infected ones. In a number of cases, the twigs bearing the shriveled needles were blackened, stunted, and slightly twisted or curved. On such twigs a second growth of needles was not produced. Although needles on all parts of the trees were infected, the new growth on the lower branches appeared to be most severely injured. The diseased needles showed an abundance of fruiting bodies protruding from both surfaces, but no spores were present.

In March, 1932, it was found that a number of the brown needles had overwintered on the twigs and that fruiting bodies were visible. An examination of this material showed excellent mature perithecia and spores. In the late spring the affected trees had developed new growth from the noninfected portions and from those twigs that were not seriously injured in 1931. The new growth, however, soon began to show yellow infection spots or entirely yellowed leaves. Infection seemed to occur particularly on the new leaves that were in contact with or in close proximity to the perithecia-bearing leaves of last year's growth.

Causal organism. The fungus on the overwintered leaves corresponds with the description of *Rehmiellopsis bohemica* Bub. and Kab., reported as causing serious injury to silver fir, *Abies pectinata,* by Bubak in Bohemia[1] and by Wilson and MacDonald in Scotland.[2] Wilson stated that, because of the disease, the planting of silver fir in Scotland had been discontinued to a large extent. He reported the disease also on *A. nobilis, A. Pinsapo, A. cephalonica,* and *A. Pindrow.* Boyce,[3] in a report of his observations of the disease in Scotland, in 1925, called attention to the fact that the only American species attacked was noble fir, *A. nobilis.*

The perithecia are single, globose, slightly conical at the upper part, and opening irregularly by the rupture of a mass of cells in the conical portion. The asci, which extrude in fascicles, are large, about 69×17 μ, clavate, with

[1] Bubak, F. Zwei neue, Tannennadeln bewohnende Pilze. Naturw. Ztschr. Forst- u. Landw. 8: 313–320. 1910.

[2] Wilson, M., and J. MacDonald. A new disease of the silver firs in Scotland. Trans. Roy. Scot. Arbor Soc. 38: 114–118. 1924.

[3] Boyce, J. S. Observations on forest pathology in Great Britain and Denmark. Phytopath. 17: 1–18. 1927.

thick walls, and a short stalk. The spores measure about 24×7 μ, are bicellular, 10 or more in an ascus, irregularly arranged, hyaline, rounded at the upper end and acute at the lower; the upper cell is broader than the lower. Paraphyses absent. No pycnidial stage has been definitely identified in the material collected. Bubak and Wilson report a pycnidial form, produced soon after infection, which they name *Phoma bohemica.* A corresponding fungus has not yet been obtained from either specimens or cultures.

Sections through the blackened twigs bearing diseased needles collected in the spring show mycelium in abundance in the leaf cushions. The hyphae gradually advance inward between the cells of the twig, being preceded by a brownish discoloration of the cell walls, a pronounced thickening of the walls and distortion of the cells, and an abnormal production of resin.

So far as known, this is the first report of the occurrence of *Rehmiellopsis bohemica* in the United States and also the first report of *Abies concolor* as a host. A similar disease has been found by the junior writer on *A. concolor* in Maine, and specimens of the disease on an unidentified species of fir were received from a correspondent in New York.

Bubak found that the young growth injured by spring frosts was most susceptible to infection. Observations in the spring of 1932 showed particularly that the Colorado firs had not been injured by frosts but were severely infected.—ALMA M. WATERMAN and MALCOLM A. MCKENZIE, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Department of Botany, Brown University.

Disease Resistance in Allium fistulosum L.—The onion variety Winterhecke, which Anderson[1] [2] found to be highly resistant to *Urocystis cepulae* Frost, has been identified by L. H. Bailey as *Allium fistulosum* L., a species reported by Zillig[3] and Anderson[2] as rather highly susceptible to smut. The Winterhecke is synonymous with the White Welsh (Ciboule blanche hâtive or Ciboule d'hiver) and very similar to the varieties Natsu-negi Nebuka, Senju-negi Nebuka, Tokyo Nebuka, and Iwatsuki, all of which belong to the same species and are highly smut-resistant. Although, under favorable conditions, smut infection in these varieties frequently averaged 10 to 20 per cent in the cotyledonous stage, it always fell to $\frac{1}{2}$ or less per cent in later counts.

[1] Anderson, P. J. Susceptibility of species of Allium to onion smut. (Abst.) Phytopath. 14: 26. 1924.

[2] Anderson, P. J. Comparative susceptibility of varieties of onions and species of Allium to Urocystis cepulae. Jour. Agr. Res. 31: 275–286. 1925.

[3] Zillig, H. Unsere heutigen Kenntnisse vom Zwiebelbrand (Tuburcinia [Urocystis] cepulae [Frost] Liro) und seiner Bekämpfung. Centbl. Bakt. II, 60: 50–58. 1923.

Another strain of *Allium fistulosum,* received under the name *A. Moly* and characterized by the production of only 2 to 3 leaves, growing at angles of about 45 to 70°, proved more susceptible to smut (45 per cent), thus supporting Anderson's finding that varying degrees of smut resistance exist in *A. fistulosum.*

The Winterhecke, White Welsh, and Nebuka strains possessed a much more vigorous root system and notably less pink root, caused by *Phoma terrestris* Hansen, than nongreen varieties, approaching maturity. The first also showed a seeming degree of resistance to mildew, *Peronospora schleideni* Unger, in that infections were confined to relatively small local areas and did not spread readily over the entire leaf surface.

Allium fistulosum is a green, hardy, bulbless onion with much variation in color of stem, being either red, yellow, or white, or all three. It is distinguished from *A. cepa* L. by the absence of lobes or teeth at the bases of the 3 interior filaments.—E. L. FELIX, A. A. Grinnell Co., Inc., and Porter & Bonney Farms Laboratory, Elba, N. Y.



ARTHUR JACZEWSKI

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ARTHUR JACZEWSKI

1863–1932

L. R. JONES[1]

Professor Arthur Jaczewski, eminent Russian mycologist and plant pathologist, completed his labors and was laid to rest February 12, 1932. For over 40 years Jaczewski had been a tireless contributor to Eurasian mycology and for 3 decades the recognized leader and teacher in Russian phytopathology. His earlier efforts culminated in the reconstruction period following the World War. He then reorganized and was the director of the laboratory for mycological and phytopathological researches in Leningrad, which later became one of the present group of national institutes, "Institut Mycologique et Phytopathologique de Jaczewski." This now continues as the central institute in this field for all U. S. S. R. Following his death his institutional coworkers prepared a memorial statement the substance of which has been incorporated in the present article.

Arthur Jaczewski was born November 3, 1863, at the family country seat,[2] "Rylkowa," in the former Russian province, Smolensk, westward of Moscow. Much of his youth was there passed with his parents in quiet rural surroundings. There he enjoyed the natural incidents of such country life, happy walks in field or forest, along with riding and hunting. Thus he

[1] The nominal writer of this sketch wishes to acknowledge the cooperation of several of Professor Jaczewski's American friends. C. L. Shear helped especially concerning Jaczewski's mycological work. Aid also has come from H. L. Bolley, H. S. Fawcett, and J. G. Dickson, who, in 1903, 1923, and 1930, respectively, like the writer, in 1930, had the privilege of visiting the Russian laboratories or experimental fields. Michael Shapovalov, also, furnished certain helpful data on Russian conditions and scientific literature. We are, however, above all, indebted to his widow, Madame Catherine Jaczewski, who with their one son, Peter, survives him. To them is owing a translation of the memorial prepared by the coworkers of the Jaczewski Institute. The son, Peter, received his later technical training in this Institute, of which he continues a staff member. He was closely associated with his father's later researches and writings.

[2] As visited by Bolley in 1903, this was still a great princely estate of the old Russian type, for the family of Arthur de Jaczewski, as the name was then written, were of the old Russian nobility. But his scientific training had strengthened his natural democratic character. He already dreamed of a future republic. He was contributing as he might in personal relations to the kindly uplift of the peasantry of the family estate and through his scientific efforts to the general betterment of rural conditions especially by the applications of modern methods of agricultural science.

early learned to love and study nature in all her forms, especially flowers and trees. The foundations were securely laid there for his later life work. As a boy he began systematic study of the native plants and started an herbarium. Quite as influential, perhaps, in the later trends was the familiarity then acquired with the crop plants of the great fields, the orchards, and gardens of Rylkowa. As was common in such early Russian families, French was the familiar home language. It was the more natural, therefore, that he spent much time in early life in France and Switzerland. Here as a youth he made long Alpine excursions, observing the plants closely. These prepared the way for his later intensive botanizing with Swiss mycologists. His interest thus became so keen, as Madame Jaczewski tells us, that "in the year 1892 he founded at the Rochers de Nage, above Montreux, the Jardin Alpin Rambertia."

His advanced studies were at the University of Berne, Switzerland. The epochal work of Woronin had naturally directed the attention of all Russian scientists to the fascinating and economically important problems of mycology. It was, however, the inspiring leadership of his Berne professor, Eduard Fischer, that contributed to young Jaczewski's early scientific advancement in special studies upon the fungi. His esteem for his early master continued to influence all his later work. In this association he began his mycological publications in Switzerland in 1889, and in 1890 his "Erysiphaceae de la Suisse" appeared. Although specializing for some years in his researches upon the mycological flora of Switzerland, he also attended special lectures at the Sorbonne and the Universities of Lausanne and Montpellier. He soon visited Italy and made an excursion into Algeria upon the mycology of which he published in 1893. In this year, also, appeared the first of his long series of papers upon Russian mycology, naturally beginning with his home province, in a catalogue of fungi of the "Gouvernement de Smolensk." In his early studies he became especially interested in the Pyrenomycetes and made a thorough study of the species occurring in Switzerland. He published in 1894–95 upon a number of different families of this group, including the Xylariaceae, Capnodiaceae, and Massariaceae, along with a more general essay on the natural classification of the Pyrenomycetes.

In 1895, in association with Professor Tranzschel, he began the distribution of "Fungi Rossiae Exsiccati." Seven fascicles of this, 350 numbers, were issued. In 1896 Jaczewski transferred his more intensive work to the Botanical Garden of St. Petersburg, there initiating a laboratory of mycology and plant pathology. With him he brought an excellent personal library and herbarium. His permanent return to Russia at the end of the last century marks the natural broadening of Jaczewski's interests concerning the bearings of mycological science upon Russian agriculture.

He had, during the summers on the Smolensk family estate, continued in intimate personal contact with rural life and agricultural problems. He held liberal political views and was of an eager progressive temperament socially as well as scientifically. It was, therefore, the natural thing that, about 1900, he recognized the great opportunity and need for organized development of plant pathology in Russia. He found the National Ministry of Agriculture sympathetic, and, in 1901, there was officially founded under this Ministry "The Central Laboratory of Plant Pathology."[3] Of this, Jaczewski was the scientific organizer and official chief. It was located in the Jardin Botanique, which, at the outset, afforded helpful library and laboratory space and other facilities.

From the first he was interested in three types of service in which this laboratory might meet the needs of all Russia. His first purpose was the continuation of his work upon the mycological survey of Asiatic, as well as European, Russia. Reference has already been made to the nature and

[3] For the details of these early developments of the "Central Laboratory" we are indebted chiefly to Jaczewski's own account: La laboratoire central de pathologie végétale du Ministère de l'Agriculture à St. Petersbourg. Bul. Soc. Mycol. France 19: 324–329. 1903.

For a more comprehensive review of the early developments in this field, the writer has relied upon the cooperation of Michael Shapovalov, of the Rubidoux Laboratory, California. Shapovalov bases the following summary upon an 18-page pamphlet (in Russian) by Jaczewski: St. Petersburg, 1911, entitled (transl.) "A sketch of the status and development of phytopathology in Russia."

This cites the few isolated Russian papers that antedated the organized developments. These included those of Woronin (1868 and later), S. M. Rozanov (1871), and the Phylloxera control committees (1880). In 1894 under the Ministry of Agriculture was organized a Bureau of Applied Botany. Under this, among other activities, was included some regional mycological surveys by Sorokin, Varlich, Dekkenbach, and Jaczewski. This decade saw notable increase in Russian publications relating to mycology and phytopathology. He cites these as follows: N. Pal'chevski (1891), Sorokin (1892), I. P. Borodin (1897), G. Varlich (1897–8), S. Rostovsev (1899), Dekkenbach (1899–1900), N. Speshnev (1900), Jaczewski (1897–99). The founding of the "Central Laboratory" came in 1901, with increased and varied activities as noted above. Jaczewski was at first director but resigned in 1905, being succeeded by A. A. Elenkin. In 1907 a new "Bureau of Mycology and Phytopathology" was formed under the Ministry of Agriculture of which Jaczewski was the director, with G. N. Dorogin and E. A. D'iakonova as associates. Especial attention was given to building up the library and herbarium that by 1911 was stated to rank with the best in Europe.

Shapovalov adds that this Bureau continued until the Revolution. Then, in 1917, it became the Division of Mycology and Plant Pathology.

Madame Jaczewski advises in correspondence that, in 1909, Professor Jaczewski made a gift of his personal library and herbarium to the bureau of which he was director. However, during the vicissitudes of the succeeding 2 decades of his life he continued to care for and enrich these from his private funds as supplementing public support, and these now constitute the basis of the valuable collections of the Jaczewski Institute, Leningrad.

importance of his continued contributions to this. The second aimed to combine especial attention to the parasitic fungi of the Russian economic plants and the related phytopathological problems, including control measures. His third concern was for educational work with the agricultural public in general, but, especially in later years, the encouragement and training of the younger Russian phytopathologists. To these purposes he faithfully devoted his time and means for the remaining 30 years of his life. His fidelity to his scientific ideals, regardless of the endless distractions and terrific strains of the wars and revolutions, is an example not to be forgotten. The size of the tasks he outlined is suggested when one recalls that, in area, the Russian territory includes 1/6 of the earth's surface and in diversity her efforts at plant culture extend from the Arctic Circle to the Caucasus and from the shores of the Baltic and Black Seas across Siberia to those of the Pacific. Yet, with tireless energy and firm grasp of detail he grappled with these duties. In 1903 Professor Bolley found him directing plant-pathological work in various regional experiment stations, and this was continuing with an able staff of associates at the time of the writer's visit in 1930. Rarely has like need found fitter scientific leadership in public service. In 1907 the earlier movement culminated, again under his direction, in the organization of the Bureau of Mycology and Phytopathology. The detailed changes in organization and personnel are shown in the preceding footnote. In this bureau were combined both research and advisory services manned by an adequate staff. Lectures in phytopathology, also, were given by Professor Jaczewski and his associates in the Institutes of Forestry and of Agronomy. These developments necessarily took much of his time and energy. Nevertheless, he continued with dauntless zeal his earlier lines of personal mycological work. While maintaining and enlarging the St. Petersburg herbarium and library, he also continued to work much during the summer at Rylkowa. Here were his simpler private laboratory and collections. In some respects his official relations enabled him, indeed, to enlarge and broaden, as well as intensify, his mycological undertakings. Immediately upon the establishment of the "Central Laboratory" in 1901 he began the preparation of a monographic treatment, 2 volumes of which were published, upon the mycological flora of European and Asiatic Russia. In 1913–17 he prepared and published in Russian 2 volumes on the determination of the fungi, containing keys and illustrations of genera and descriptions of the species. To the revision and enlargement of this he devoted time throughout the remaining years of his life. Two months before his death appeared the first part of the third edition of this work, covering the Phycomycetes. Besides such more inclusive works, he published many special articles on fungi and was very deeply interested in the problems of phylogeny. His latest paper on this

subject was published in Phytopathologische Zeitschrift in 1929. Professor Jaczewski was a man of unusual powers of memory and had a wide and thorough acquaintance with nearly all groups of the fungi. He held conservative ideas concerning genera and species and his leadership has laid sound foundations for future developments of mycology in Russia.

Meanwhile numerous publications, including manuals, appeared treating directly of phytopathology especially as applied to the special crops and conditions in Russia. From the outset of his personal leadership in Russian plant pathology to his very last days he strove ceaselessly to serve his country through science and to inspire his numerous pupils to do likewise.[4] It is unnecessary to dwell upon the hardships that he, like many of the Russian contemporaries, experienced during and after the Revolution. Perhaps the solace of determined work helped to sustain. At first financial support from the Government was lacking, and, of course, his private resources were gone. As the new programs took form he struggled to continue as he might his precious research laboratory in Leningrad. He led in 1920 in founding the Mycological Society of Russia. He was one of the initiators soon after the Revolution of a High School of Phytopathology and Applied Zoology, where he lectured. In 1921 he received the support of the new government in a journey of 5 months to the United States. He visited the various phytopathological centers in Washington and the several States, making warm friendships and gaining inspiration and information for the development of his later phytopathological projects. Probably there was no happier period in the last decade of his life. During this visit to the United States he became a patron of the American Phytopathological Society. When Fawcett visited him in 1923 he reports him stimulated by the memory of his visit to this country, working personally with characteristic energy and enthusiasm in spite of lack of adequate means or assistance. As the new government gained momentum these were increasingly granted. In 1927 his scientific friends and associates honored him on the 35th jubilee of his scientific career. In recognition of his services the Government of the U. S. S. R. then applied his name to the mycological

[4] Concerning Jaczewski's national service we are permitted to quote from a personal letter recently received by the writer from Dr. N. I. Vavilov, President of the Academy of Agricultural Sciences, U. S. S. R., of which the Jaczewski Institute is a part:

"Until Dr. Jaczewski's last days he was working, dictating his book, 'Phytopathology.' The last day before his death he sent me a letter, as President of the Academy, outlining a new scheme for Phytopathological Scientific Service. It is difficult, indeed to appreciate fully the greatness of his work. He was the founder of the Phytopathological Service and the teacher of all the phytopathologists of our country. I was myself his pupil. It was in his Institute that I did my work on immunity of plants to infectious diseases."

laboratory and later elevated it in rank to a national "Institute." When the writer visited this Institut Jaczewski in 1930 he found it well housed in the former palace of the Greek Embassy, library and herbarium in excellent condition, laboratories filled with varied activities, and the center of nation-wide research and educational influence. There was a staff, including some at other stations, of perhaps 70 trained workers and an annual budget of some 400,000 rubles. It was evident, however, that he was even then reaching the limit of his bodily endurance, although not of his unyielding will to work.

Thus ended his fruitful career. Dominated to the last by worthy ideals of service through science, giving all he had in zealous pursuit of these, undaunted, undistraught, let happen what might. And, withal, as his intimate associates and family knew him, ever the same courteous, considerate gentlemen; pure-minded, open-hearted, honest seeker of right and truth, interested in art, music, and all good things; "kind and amiable to everyone," "respected, admired, and loved by friends, associates and pupils." The many friends made during his American journeys join in these tributes from his coworkers.

PATHOGENICITY AND ENVIRONAL STUDIES ON VERTICILLIUM HADROMYCOSIS

W. V. LUDBROOK[1]

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INTRODUCTION

The literature on Verticillium hadromycosis is very extensive and scattered. General surveys have been published by Van der Meer (16) in 1925 and by Rudolph (14) in 1931. The latter paper is thought to include all references worthy of note up to the early part of 1929. Wollenweber (17) and Berkeley, Madden, and Willison (2) in papers published in 1929 and 1931, respectively, give particular attention to the history of the taxonomic treatment of the vascular Verticillia in connection with reports of their own work on this aspect. As each phase of the writer's own work is taken up, a résumé of previous work on that phase will be given.

CAUSAL ORGANISMS

History of Taxonomic Treatment

Reinke and Berthold (13) were the first to isolate, describe, and name the causal organism of Verticillium hadromycosis. They apparently did not realize the possibility of taxonomic confusion arising from the variability of the pathogen. Their diagnosis of the species *V. albo-atrum* R. and B. undoubtedly includes the type forming dark hyphae but leaves some room for doubt as to whether it was intended to include also the type forming microsclerotia. Klebahn (12) isolated a vascular Verticillium forming microsclerotia, which he considered to differ from Reinke and Berthold's description of *V. albo-atrum* enough to warrant the establishment of a new species, *V. dahliae* Klebahn. This included vascular Verticillia forming microsclerotia, the older designation being reserved for types forming dark hyphae and not microsclerotia. Van der Meer (16), Dufrenoy (8), and Berkeley, Madden, and Willison (2) uphold this viewpoint, pointing out that the difference in morphology may be accompanied by a difference in

[1] The writer wishes thankfully to acknowledge his indebtedness to the following: (1) Dr. J. C. Walker, for suggesting the problem and for his advice and encouragement throughout the investigation; (2) other members of the Department of Plant Pathology, University of Wisconsin, for assistance in various ways; (3) the many workers who sent cultures of *Verticillium* spp. on request; (4) the University of Wisconsin, for granting the writer an honorary fellowship in plant pathology during the investigation, which was carried on at this institution; (5) the Council for Scientific and Industrial Research of the Australian Government, whose award of a research studentship to the writer made the investigation possible.

pathogenicity. Carpenter (6), Wollenweber (17), and Rudolph (14) consider that Reinke and Berthold's description was meant to include both types, which together with several other species of vascular Verticillia should probably be regarded as varieties or forms of *V. albo-atrum* R. and B. The papers of Rudolph (14), Wollenweber (17), and Berkeley, Madden, and Willison (2) may be consulted for more complete reviews of the literature on the question of taxonomy.

Whilst the writer has not studied the question of the validity of species of vascular Verticillia other than *Verticillium albo-atrum* and *V. dahliae*, he has no hesitation in supporting the views of Klebahn, Van der Meer, and Berkeley, Madden, and Willison on the desirability of treating these two as separate species. It was found that 45 out of 46 cultures, collected from widely different hosts and localities, could be readily and definitely classed under one or the other of the two species as defined by Klebahn. The remaining culture did not form any dark mycelium[2] whilst under the writer's observation and, therefore, could not be assigned to either of these species.

Classification of Pure Cultures Studied

The cultures of vascular Verticillia studied in the present investigation are listed in table 1. The collection comprises 46 cultures, coming from 13 States of the United States, 4 European countries, and 4 States of Australia. They were isolated from 20 different host species belonging to 17 genera. The cultures forming dark mycelium have been classified according to the views of Klebahn and Van der Meer, *i.e.*, all those forming definite microsclerotia have been placed in the species *Verticillium dahliae* Klebahn, and all those forming dark hyphae without microsclerotia have been classified as *V. albo-atrum* R. and B. Figure 1 illustrates the microscopic appearance of dark mycelium from a typical culture of *V. dahliae* and 2 types of *V. albo-atrum*. The one culture that formed no dark mycelium (No. 5 in Table 1) at first formed no verticillate conidiophores, though it formed conidia fairly readily on thin unbranched or irregularly branched conidiophores. It was not until near the end of the investigation that a few verticillate conidiophores were found in a portion of an agar plate culture that had been macerated with a scalpel. As the writer was not until then satisfied that it was a Verticillium, it was not given special attention. Its peculiarities were not altered by three consecutive plant passages. The symptoms produced in egg-plant were similar to those caused by *V. dahliae* or *V. albo-atrum*. Berkeley has used the term "*album* group" to distinguish vascular Verticillia forming no dark mycelium. In a recent letter to the writer he states that many cultures

[2] The term "dark mycelium" is used here to include both microsclerotia and dark hyphae not forming microsclerotia.

TABLE 1.—*List of pure cultures of Verticillium spp. studied, classified according to species and host*

No.	Host	Locality	No.	Host	Locality
V. albo-atrum			*V. dahliae* cont'd.		
12	Tomato	Oregon	2	Cotton	Illinois
27	"	England[a]	13	"	Tennessee
35	"	Wisconsin	45	"	Queensland
14	Potato	England	46	"	"
17	"	United States	18	African daisy	Iowa
31	"	Maine	40	Aster	Germany
38	Hop	England	41	"	"
39	Cucumber	Ohio	21	Chrysanthemum	Washington
			22	"	England
"album" type			4	Dahlia	Illinois
5	Black raspberry	Oregon	3	Lupin	"
			15	Snapdragon	United States
V. dahliae			11	Apricot	California
1	Eggplant	Wisconsin (Racine)	42	"	Victoria
20	"	" (Madison)	29	Aralia	New York
24	"	" (Racine)	32	Barberry	"
25	"	"	30	Daphne	Europe
34	"	Pennsylvania	16	Elm	United States
7	Tomato	Ohio (Toledo)	28	"	Delaware
8	"	" (Cleveland)	33	"	New York
9	"	California	23	Maple	Rhode Island
26	"	Guernsey, C. I.	10	Raspberry	California
43	"	South Australia	37	"	England
44	"	"	6	Sumac	Illinois
19	Potato	Wisconsin	48	Host unknown	New South Wales

[a] Dark hyphae longer and coarser, and darkened bases of conidiophores more conspicuous than in the other cultures of *V. albo-atrum.* (Fig. 1, C.)

thus classified by him have subsequently formed dark mycelium under special cultural conditions, *e.g.,* when grown on potato plugs. Possibly on further study culture No. 5 could be referred to *V. dahliae* or *V. albo-atrum,* but for the purposes of this paper it will be tentatively designated "*album* type."

A given isolation of the fungus often varied as to the rapidity and profusion with which dark hyphae or microsclerotia were formed under varying conditions of growth and length of time in artificial culture. This has been the usual experience of workers with this group of fungi. The writer's classification into species is based finally upon observations of cultures on potato-dextrose-agar slants, which had been kept at about 12°[3] without transfer for 12 months. The agar in these cultures had dried

[3] All temperatures are in degrees Centigrade.

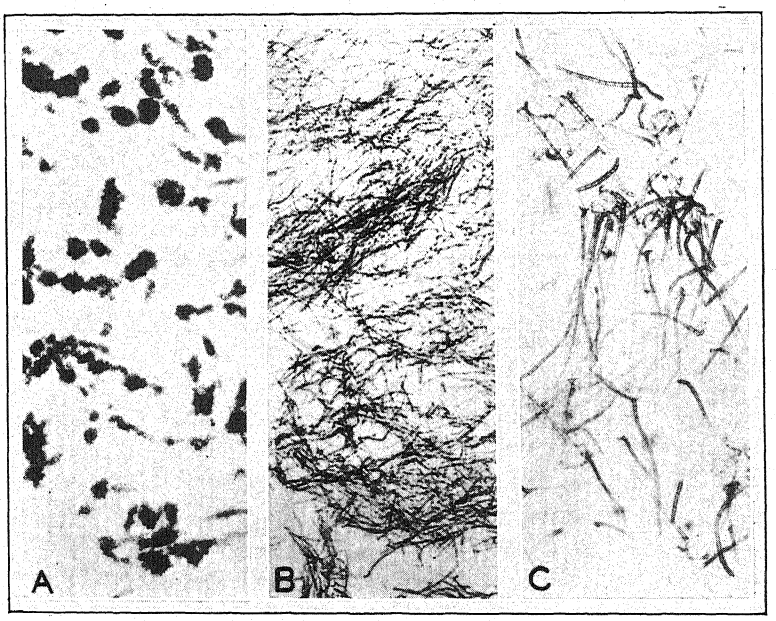


FIG. 1. A. *Verticillium dahliae*, showing typical microsclerotia with attached chains of chlamydospore-like cells. B. *V. albo-atrum*, showing typical dark hyphae. C. *V. albo-atrum*, culture No. 27 (see Table 1, footnote a). All unstained. ×60.

down to a tenth or less of its original bulk, but the cultures were all readily viable, as was proved by transfer. Several younger transfers were also observed in each case. No difficulty was experienced in making a sharp distinction between the two species in any of the cultures dealt with, except No. 5. When agar slants were inoculated with a spore suspension obtained by mixing suspensions from single-spore cultures of No. 25 (*Verticillium dahliae*) and No. 35 (*V. albo-atrum*) each species produced its characteristic dark mycelium in the resultant mixed growth. It is therefore thought that if any of the cultures had contained both types, this fact would have been evident on microscopic examination. In no case were both types found by microscopic examination in the same culture, though single-spore isolations were made in only a few instances.

At least 12 of the cultures (8 of *Verticillium dahliae* and 4 of *V. albo-atrum*) produced no dark mycelium until the transfers were several weeks old. Such cultures grown on prune-agar plates at 22° had not produced dark mycelium at the end of 3 weeks, while others produced it in 1 week or less. Cultures 1, 3, 13, and 20, under the same conditions, produced dark mycelium only in small sectors of the colonies. Since these cultures were not from single-spore isolations, the sectors may have been due to a

mixture of races. The white portions of these colonies later produced dark mycelium. The cultural variability of these 2 species, and more particularly of *V. dahliae,* is such as to suggest the possibility of hyphal fusions between different races being responsible for the variations, as has been proposed by Brierley (5) for similar variability in other fungi. In a paper entitled "The Mechanism of Variation in Imperfect Fungi; *Botrytis cinerea*" accepted for publication in Phytopathology (1932) Hansen and Smith mention *V. albo-atrum* as one of several fungi in the case of which they have evidence indicating that variation may be due to "nuclear assortment" resulting from hyphal fusions. Thus each multinucleate conidium may give rise to more than one genotype.

In spite of these variations, no culture producing microsclerotia was ever observed to produce dark hyphae (except short, rounded or irregular cells closely associated with the microsclerotia) nor was any culture producing typical dark hyphae ever observed to form microsclerotia. It was, therefore, considered expedient for the purposes of this paper to classify the collection of cultures into the two simple species *Verticillium dahliae* Klebahn and *V. albo-atrum* R. and B., with the exception of No. 5, tentatively designated "*album* type." The desirability of treating *V. dahliae* and *V. albo-atrum* as distinct species is affirmed by some authors and questioned by others, as previously mentioned. In the hope of throwing further light upon this problem, the writer obtained as representative a collection of cultures as possible in order to compare the pathogenicity and temperature relations of (1) members of the two species as separate groups and (2) different isolations of the same species.

Geographic Distribution of Species

From table 1, *Verticillium dahliae* is seen to include over 80 per cent of the cultures obtained. The numbers dealt with are, of course, much too small to warrant the drawing of definite conclusions with regard to the relative distribution of the 2 species, but it may be pointed out that all the cultures of *V. albo-atrum* came from northern United States and England, where the summers are relatively cool, while none was obtained from the warmer regions in southern United States or Australia. *Verticillium dahliae,* on the other hand, appeared to be widely distributed in both warmer and cooler regions. This agrees with the findings of previous workers and the writer, that *V. dahliae* has a higher maximum temperature than has *V. albo-atrum.*

Temperature Relations of Verticillium dahliae and V. albo-atrum in Culture

Bewley (4) stated that "the minimum, optimum, and maximum temperatures for growth in pure culture of the strains of *V. albo-atrum*

utilized for the inoculations were 4.4° C., 23.3° C., and 30° C. respectively," but he did not indicate whether the cultures used were of the *V. dahliae* or *V. albo-atrum* type. It is evident from his description of his collection of cultures that both types were included by him under the simple designation *V. albo-atrum,* as was the case with most of the earlier workers. Chaudhuri (7) gives 10°, 22.5°, and 27° as the minimum, optimum, and maximum for the culture he used, which he describes simply as *V. albo-atrum.* Edson and Shapovalov (9) and Wollenweber (17) have found the maximum temperature for certain cultures of vascular Verticillia, not producing microsclerotia, to be about 30° to 31°, while the maximum for cultures producing microsclerotia was distinctly higher, in some cases 35° to 36°. The former workers used one culture of each type, and the latter 2 asclerotial and 8 sclerotial types, all of which were classified by these authors as *V. albo-atrum* or varieties of the same.

The results of most temperature studies by previous workers agree on the point that, in general, the optimum temperature for growth of vascular Verticillia in culture is in the region of 23°, though Pötschke in a brief note gives 28° for a Verticillium causing hadromycosis in horse-radish. The maxima reported vary from 27° to 37°. Where the type of fungus used is described, cultures of the *V. albo-atrum* type generally appear to have produced little or no growth at 30°, while those of the *V. dahliae* type grew fairly well at 30° and in some cases at higher temperatures. The writer's temperature studies in culture were, therefore, concerned chiefly with the upper end of the temperature range in order to determine whether or not the higher maximum were peculiar to the *V. dahliae* group.

Experimental Data

Each culture was inoculated by mycelial transfer in 3 places on a potato-dextrose-agar plate and grown at 28°. The diameter of each colony was measured at the end of the 3rd and the 10th days after inoculation. Thus, the average total increase in diameter over a period of 7 days was calculated for each culture. The values so obtained for the 8 cultures of *Verticillium albo-atrum* averaged 5 mm. (maximum = 6, minimum = 4); for the 37 cultures of *V. dahliae,* the average was 22 mm., the maximum 28, and the minimum 14. At 30°, none of the 8 cultures of *V. albo-atrum* made any appreciable growth. Twelve cultures of *V. dahliae* were grown at 30° and gave average total increases of colony diameter ranging from 3 to 15 mm. in 7 days at 30°. The culture giving 15 mm. increase at 30° was grown at 22° and 28°, 3 colonies at each temperature, in 2 separate experiments. The increase in colony diameter averaged 30 mm. at 22° and 32 mm. at 28° on both occasions. Two typical cultures of each species grown at 22° gave increases of colony diameter ranging between 24 and 29

mm. in 7 days. With regard to the reliability of increase in colony diameter as a measure of growth, Chaudhuri (7) states that he found it reliable for any one culture grown at different temperatures under conditions identical in other respects. It probably does not give an accurate comparison of the growth rates of different cultures even under identical conditions. Therefore, the different amounts of increase in colony diameter of the different cultures of *V. dahliae* when grown at the same temperature do not necessarily indicate different amounts of actual growth, *i.e.*, production of fungal material. As regards the comparison of *V. dahliae* with *V. albo-atrum* at 28°, the difference in average increase of colony diameter is thought to be too great to admit of the possibility that it was not accompanied by a difference in actual growth rate, although the difference may not have been so large as would appear from the measurements of colony diameter. Furthermore, as previously stated, there was relatively little difference between the rates of increase of colony diameter of representatives from each species at 22°.

Thus, the writer's study of a collection of cultures more extensive than those used by previous workers shows that the upper limit for *Verticillium albo-atrum* lies between 28° and 30°, while *V. dahliae* is generally characterized by a distinctly higher maximum temperature for growth in culture. A corresponding difference in the maximum temperatures for disease production will later be pointed out. The optimum temperature for *V. albo-atrum* (in the broader sense) is usually stated in the literature as about 23°, but 1 culture of *V. dahliae* gave slightly more growth at 28° than at 22°, indicating that in some cases the optimum range of temperature extends higher than has hitherto been realized, except for the report of Pötschke mentioned above.

PATHOGENICITY STUDIES

Rudolph (14) summarized the cross inoculations recorded as having given positive results up to 1928 in a table including Verticillium cultures from at least 25 different hosts. Each of these cultures had been inoculated into one or more of 27 hosts other than that from which it was isolated. Negative results were obtained in many cases, but they cannot always be regarded as conclusive, due to the marked and imperfectly understood influence of environment on the disease. It would seem that there is little or nothing approaching strict biologic specialization amongst the vascular Verticillia, but important differences in pathogenicity between *V. dahliae* and *V. albo-atrum* have been recorded. It has been shown by Van der Meer (16) and by Berkeley, Madden, and Willison (2) that cultures of *V. albo-atrum* are often much more pathogenic than those of *V. dahliae* to certain hosts, particularly tomato, potato, and cucumber. The latter

authors produced definite disease in cucumber with each of 5 cultures of *V. albo-atrum,* but none of 32 cultures of *V. dahliae* produced any symptoms on cucumber. Seedlings of *Dahlia* sp. were affected by one culture only, a type culture of *V. dahliae* Kleb. Tomatoes were severely affected by all 5 cultures of *V. albo-atrum,* while different cultures of *V. dahliae* varied from strong to very weak parasitism on this host.

Susceptibility of Various Hosts to Verticillium Hadromycosis

The following field experiments were conducted by the writer during 1930, in which 15 plant species previously reported as susceptible to Verticillium hadromycosis were used. The aims were to compare the susceptibility of different hosts to the disease and to see whether any evidence of biologic specialization could be found. No such evidence was found. The tomato and potato, which gave negative or doubtful results in these experiments, later proved much more susceptible in greenhouse experiments, so that all the negative results obtained in these field experiments are possibly due merely to conditions unfavorable for disease development. The results are therefore thought to be of value only as an indication of the relative susceptibility of the hosts used under the conditions of the experiment, which were as far as possible uniform, except for the 2 methods of inoculation employed. The temperatures in the field were approximately as given in figure 8.[4] Inoculations were performed during the first 2 weeks in June, except those on the potato, sets of which were planted in inoculated soil during the 3rd week in May. Where external symptoms appeared, they became evident within a month of inoculation. Inoculations of herbaceous plants were made by mixing 40 gm. of corn-meal-sand inoculum with the soil into which each seed or seedling was planted. Woody plants were inoculated by introducing spore suspension or mycelium into wounds on individual shoots. The wounds were then covered with nurseryman's tape. Aseptic conditions were not maintained, but controls were run and reisolations made from diseased plants from tissue at least 5 cm. from the point of inoculation. No sign of disease was ever observed in the controls except in 6 eggplants each of which was within 3 ft. of plants heavily inoculated with corn-meal-sand inoculum. In most cases results are based on inoculations of 20 to 40 plants. Nine cultures of *V. dahliae,* isolated from 8 different hosts, and 1 culture each of *V. albo-atrum* and the *"album"* type were used. Eggplants, peppers, potatoes, tomatoes, and cucumbers were inoculated with the majority of these cultures; the remainder of the hosts were all inoculated with a culture of *V. dahliae* from eggplant.

[4] A full discussion of the data in this figure will be found under the heading, "Environal Studies—Temperature Relations in the Field."

Under the conditions of the experiment, the eggplant appeared to be the most susceptible of the hosts used; cotton, Antirrhinum, and okra were also severely affected by *Verticillium dahliae*. Individual shoots of garden rose suffered rapid withering of all the leaves above the point of wound inoculation with *V. dahliae;* milder symptoms (slow discoloration and withering of the leaves just above the wound) were produced by *V. dahliae* in shoots of wild sumac (*Rhus typhina*), wild blackberry and wild raspberry. Noninoculated wounds in control shoots caused no symptoms. Potatoes and tomatoes displayed no external symptoms of disease when inoculated with *V. albo-atrum* or *V. dahliae,* though successful reisolations were made in some cases from the xylem of the stem just above ground level. No symptoms at all were displayed by sweet pea, lupin, pepper, or cucumber[5] inoculated with *V. dahliae.*

Pathogenicity of Verticillium dahliae and V. albo-atrum. Greenhouse Experiments

Potted eggplants, tomatoes, and potatoes were inoculated with cultures of *V. dahliae* (No. 25) and *V. albo-atrum* (No. 35), in some cases through wounds at the base of the stem and in others by mixing corn-meal-sand inoculum with the soil. Ten plants of each kind were subjected to each method of treatment. Ten controls of each were grown in soil containing sterilized inoculum, and 10 were wounded at the base of the stem. The plants were kept at about 20°. In each case symptoms of disease became evident in the plants inoculated with *V. albo-atrum* a week or more earlier than in those inoculated with *V. dahliae,* and the former were much more severely diseased than the latter. The difference was less conspicuous in the eggplants, in which marked symptoms were caused by both species of Verticillium. Nevertheless, *V. albo-atrum* caused a reduction of about 40 per cent in the dry weight of the inoculated plants, as compared with about 10 per cent in the case of *V. dahliae,* after 6 weeks' growth. In the tomatoes and potatoes the only symptom caused by *V. dahliae* was a slight retardation of growth without wilting or withering of the leaves, while *V. albo-atrum* produced a much greater stunting and also wilting and death of the lower leaves of inoculated plants.

These experiments were repeated three times with eggplant and tomato and twice with potato, similar results being obtained each time. They were also repeated with cultures 14 (*Verticillium albo-atrum*) and 44 (*V. dahliae*), which showed a like difference in pathogenicity. As will be described under the heading "Environal Studies," culture No. 25 (*V. dahliae*) failed to produce definite symptoms of disease in tomato plants at

[5] Successful inoculations of cucumber with *V. albo-atrum* were later performed in the greenhouse at 20°. *Verticillium dahliae* produced no effect.

soil temperatures between 16° and 30°, while No. 35 (*V. albo-atrum*) produced marked symptoms from 16° to 26° (Fig. 7). The plants in this soil-temperature-tank experiment made much more vigorous and rank growth than did the potted plants described above.

In several further series of inoculations upon potted tomato plants at mean temperatures between 18° and 23°, all the available cultures were tested for pathogenicity. The plants were grown for 4 to 6 weeks after inoculation, which was found to be ample time for the development of marked symptoms in plants inoculated with pathogenic cultures. Six, 7, or in most cases 9 plants were inoculated with each culture in each series. Inoculations were performed in most cases by pouring an aqueous suspension of a macerated potato-dextrose-agar culture of the fungus around the roots when potting the seedlings. In 2 series, wound-inoculations into the stems were made, and, in another, corn-meal-sand inoculum was mixed with the soil. The severity of the symptoms and the average dry weight of stems and leaves were recorded for the plants inoculated with each culture in each series of inoculations. Not all of the cultures were tested in more than one series of inoculations, but representative cultures of each species were tested in from 2 to 5 different series and gave consistent results. A standard culture of each species was included in every series.

TABLE 2.—*Summary of results of pathogenicity experiments with potted tomato plants. The weights of inoculated plants given are relative to those of the noninoculated plants, which were taken as 100 in each series.*

Species of fungus	Symptoms moderate to severe			Symptoms slight or absent		
	Number of cultures	Total number of trials[a]	Average dry weight of tops	Number of cultures	Total number of trials[a]	Average dry weight of tops
Verticillium dahliae	9	16	78	28	35	107
V. albo-atrum ...	6	14	59	2	6	98
"*album*" type				1	4	90

[a] This number is obtained by writing down for each culture the number of separate series of tests in which that culture was included (varying from 1 to 5 for different cultures) and totalling these figures.

The results are summarized in table 2. Six out of 8 cultures of *Verticillium albo-atrum* were severely pathogenic to tomato, causing conspicuous mottling with yellow, wilting and withering of the lower leaves, also marked reduction in the dry weights of stems and leaves as compared

with those of noninoculated plants, the latter being taken as 100 in each series. The other 2 cultures of *V. albo-atrum* (Nos. 17 and 38) displayed little or no pathogenicity. Nine out of 37 cultures of *V. dahliae* produced marked to severe disease symptoms and reductions in weight, though on the average less severe than those due to *V. albo-atrum*. The remaining 28 cultures of *V. dahliae* and the one culture of the *album* type displayed little or no pathogenicity. Two single-spore cultures of *V. dahliae* that were strongly pathogenic to the rather weak plants used in one series proved to be relatively less pathogenic to the more vigorous plants used in the remainder of the experiments, as compared with the typical *V. albo-atrum* cultures. Thus, the relative severity of disease produced by a given culture may vary according to the general vigor of the host plants. The above results agree with those of previous workers except that 2 cultures of *V. albo-atrum* were found to exhibit little pathogenicity to tomato. These cultures were somewhat atypical in cultural characters, and one had been in culture for an unknown length of time. However, it is not considered that variation in the age of the cultures used seriously vitiates these results. Most of them were isolated during the latter half of 1930. Culture No. 31 (*V. albo-atrum*) had been grown on artificial media continuously since 1924, and No. 7 (*V. dahliae*) since 1927. Each was one of the most pathogenic of its own species.

Field Experiments

Field experiments were conducted in 1930 and 1931 to investigate the possibility of differences in pathogenicity between different cultures of *V. albo-atrum* and *V. dahliae*. In 1930, the hosts used were eggplant, tomato, potato, cucumber, and pepper. One culture of *V. albo-atrum* and 8 of *V. dahliae* were used, 50 gm. of corn-meal-sand inoculum being mixed with the soil in the bottom of the hole into which each seedling was transplanted. Twenty plants of each host were inoculated with each culture. Ten noninoculated plants of each host grown between each pair of inoculated strips made good growth and were free of disease. The temperatures during the growing periods of 1930 and 1931 are shown in figure 8.

Of the inoculated plants in 1930, the potatoes, cucumbers, and peppers showed no signs of disease, whatever. The tomatoes were unaffected except for slight vascular discoloration at the base of the stem produced by 6 out of 9 cultures, including *Verticillium albo-atrum*. At the end of the growing period but before the plants had begun to die off, the fungus was reisolated from 13 out of 30 such tomato plants from the stem at least an inch above ground level. Nearly all the eggplants inoculated were stunted to about ⅔ the size of the noninoculated plants; their lower leaves became mottled with yellow, wilted, and dried up. There was no appreciable dif-

ference in severity of disease between the plants inoculated with the different cultures, though the cultures all showed marked differences in appearance when grown on agar plates.

In 1931 similar experiments were carried out in which 4 cultures of *Verticillium albo-atrum* and 3 of *V. dahliae* were used. The same general procedure and hosts were used, except that peppers were omitted and the quantities of inoculum were varied between 20 and 80 gm. per plant without visibly influencing the severity of the disease. In recording the results, an arbitrary scale of severity was adopted, ranging from 0 (plant killed without making a significant amount of growth after inoculation) to 5 (plant unaffected). The eggplants showed much less disease than in 1930. Towards the end of the growing period, each inoculated eggplant was assigned one of the figures of the above scale. The average values thus obtained for the lots inoculated with *V. albo-atrum* cultures were 4.5 (culture No. 39), 3 (No. 14), 3.5 (No. 35), and 4 (No. 27) and for those inoculated with *V. dahliae* cultures, 5 (No. 44), 3.5 (No. 25), and 4.3 (No. 21). The tomatoes were unaffected, except for slight vascular discoloration at the base of the stem in about a half of the plants inoculated with culture No. 14 (*V. albo-atrum*). In the potatoes, one culture of each species (Nos. 21 and 35) produced slight vascular discoloration in the base of the stem; the remainder had no effect.

These results cannot be held to afford satisfactory evidence for or against differences in pathogenicity, in view of the small differences and amounts of disease observed. The experiments are described mainly because they indicate that symptoms of Verticillium hadromycosis may partially or wholly fail to develop in artificially inoculated field-grown eggplants, tomatoes, and potatoes under mean-temperature conditions found favorable for disease of these hosts when grown in soil-temperature tanks. These experiments will be further discussed from the environal viewpoint after describing the soil-temperature tank experiments.

ENVIRONAL STUDIES

Literature

Temperature appears to be the most important environal factor influencing the severity of this disease (Rudolph, 14). Studies of temperature effect on growth of the fungus in culture have already been considered, and attention will here be confined to observations on the effect of temperature on the occurrence and severity of the disease, treating firstly of greenhouse and secondly of field studies. The effect of soil moisture also will be considered in connection with the greenhouse studies on eggplant.

Bewley (3, 4) made observations in commercial glass houses, which indicated that temperatures between 15.5° and 24° were favorable for

the disease in tomato, the optimum being about 22°. The disease was partially counteracted by shading. Schoevers (15) gives 25° as approximately the maximum temperature at which the disease is likely to be evident in tomatoes. Alexander (1) gives a brief preliminary report of soil-temperature-tank experiments with tomatoes. Sterilized soil was inoculated with *Verticillium albo-atrum*. Severe disease occurred at soil temperatures between 12° and 24°. At 28° plants showed only slight symptoms and made as good growth as the noninoculated plants.

Soil moisture is recorded in the last-mentioned report as having no influence on the disease. Many opinions based on observational evidence of moisture relations have been published. Extremes of both high and low moisture have been reported as aggravating the disease in various hosts. Bewley (3) considers that heavy watering increases the severity of the disease in tomatoes. Van der Meer (16) reports as follows: "Some pot experiments with the potato variety 'Eigenheimer' inoculated at the stem base with *V. albo-atrum* or *V. dahliae* (potato) were arranged as follows. One set of plants was kept moist under glass, a second was watered every day, and a third was placed under a penthouse and kept as dry as possible. It appeared that the development of the disease was much more rapid in drought, and that under very moist conditions the plants suffered but little from the disease."

Temperature and Moisture Relations Under Controlled Conditions

Methods. Standard Wisconsin soil-temperature tanks were used, the soil temperature being controlled to within half a degree Centigrade. The air temperature was maintained at or above a constant minimum, variations being recorded by a thermograph. Mean daily and weekly air temperatures were determined by averaging the thermograph readings at intervals of 2 hours throughout the period in question. Soil moistures were maintained as constant as possible by periodical weighing and addition of the required amount of water. At the end of the first experiment with eggplant a test of representative soil samples indicated that the average moisture content of each can had not changed by more than about 2.5 per cent of the moisture-holding capacity from the desired moisture content, though there was a tendency for the water to accumulate at the bottom of the can in the case of 85 or 95 per cent saturated soil. Except where the effect of higher or lower soil moistures was being investigated, the soil was kept 65 per cent saturated with water.

The cultures used were as follows, except where otherwise stated. The *Verticillium dahliae* culture chiefly used (No. 25 in Table 1) was from an eggplant at Racine, Wisconsin; the *V. albo-atrum* culture chiefly used (No. 35 in Table 1) was from a tomato plant in a greenhouse near Milwaukee, Wisconsin. Both were isolated by the writer in November, 1930. In each

case, 1 of a number of single-spore isolations was chosen and used throughout this series of experiments, except that cultures from other sources were used for comparison in certain specified cases. The fungus was grown on corn-meal sand for about 10 days: about 50 gm. for eggplants and 80 to 160 gm. for potatoes and tomatoes were mixed with the top third of the soil in each inoculated can. A like amount of sterilized inoculum was mixed with the soil in each control can. Nonsterilized soil was used throughout all the tank experiments to be described, and no sign of disease was found in any noninoculated plant.

The varieties of host plants used were Black Beauty eggplant, Bonny Best tomato, and Bliss Triumph potato. Three seedlings about 3 in. high were planted in each can in the case of eggplant and tomato; 2 pieces of potato tuber were planted, only 2 shoots per can being allowed to develop. The cans were 6 by 10 in. The time of first appearance of definite symptoms (paleness and flaccidity of the distal or sometimes lateral margins of the lower leaves) was noted for each can. All the plants in each can usually showed symptoms within a day or two of one another, except in the case of the potatoes, where the time for each plant was recorded. Unless otherwise stated, the experiments were continued until the noninoculated plants were about to become too large for healthy growth in the cans. In order to obtain a roughly quantitative expression of the results, the plants in each can were then severed from the roots at ground level, dried for 24 hours at 100° C., and weighed. The weights per can of noninoculated and inoculated plants were tabulated for each experiment. The reductions in the weights of the inoculated plants were taken as roughly indicative of the relative severity of the disease and were expressed as percentages of the weights of the corresponding noninoculated plants, to give a uniform basis for comparison. It must be emphasized that these weights and percentages are in every case liable to a high experimental error, due to the small numbers of plants used. They are employed merely to enable the results of these particular experiments to be stated more explicitly than would otherwise be possible.

EGGPLANT HADROMYCOSIS

Soil temperature and moisture relations at 19° to 22° air temperature. The 1st experiment was conducted during the 7 weeks beginning February 7, 1931, and the 2nd during the 6 weeks beginning April 9, 1931. The mean air temperature was 19° during the first period and 22° during the second. The soil temperatures and moistures used are enumerated in table 3,[6] together with the weights of noninoculated plants and percentages

[6] In addition to these, a soil temperature of 12° and a soil moisture of 35 per cent of saturation were tried in the first experiment. Severe disease occurred under these conditions, but the uninoculated plants made so little growth that the results will not be further considered.

TABLE 3.—*Influence of soil temperature and moisture on (1) dry weights[a] of noninoculated eggplants, (2) reductions[b] in weights of eggplants inoculated with Verticillium dahliae in 1st and 2nd tank experiments*

Soil moisture in percentage of moisture-holding capacity	Experiment No.	Soil temperatures (mean air temperature = 19° for 1st and 22° for 2nd experiment)											
		16°		20°		24°		28°		30°		32°[c]	
		Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants
45	1	2.2[a]	70[b]	6.3	86	8.6	85	5.6	80			5.3	26[c]
	2					6.0	80	5.4	72	5.6	43	5.1	−6[b]
65	1	2.4	54	8.5	82	9.7	74	12.7	80			10.6	20
	2					11.2	64	12.3	64	11.9	68	10.0	17
85	1	1.7	29	7.1	73	11.0	75	14.5	61			13.0	21
	2					13.0	65	14.7	37	21.7	47	16.0	−2
95	2					16.3	73	19.7	49				

[a] Each weight given is the total weight in grams of the 3 plants in 1 can (excluding roots) at the end of 7 weeks' (1st experiment) or of 6 weeks' (2nd experiment) growth.

[b] The amounts by which the dry weights of the inoculated plants fell short of those of the corresponding noninoculated plants are expressed as percentages of the latter. A negative percentage, therefore, signifies that the inoculated plants weighed more than the noninoculated plants.

[c] There were no symptoms of disease at 32°, and the reductions in weight that occurred in some cases are thought to be attributable to experimental error.

of reduction in the weights of inoculated plants. *Verticillium dahliae* (No. 25) was used in both experiments. Space permitted the inclusion of only one inoculated and one noninoculated can in each experiment for each set of conditions, but the results of the 2nd experiment satisfactorily confirmed those of the 1st with regard to temperature, and subsequent comparative experiments with other cultures of Verticillium gave abundant confirmation of results at the critical temperatures.

The incubation periods in the 1st experiment varied from 9 to 16 days and in the 2nd from 16 to 26 days, the increase in the 2nd experiment probably being due to the greater vigor of the plants. While the data on

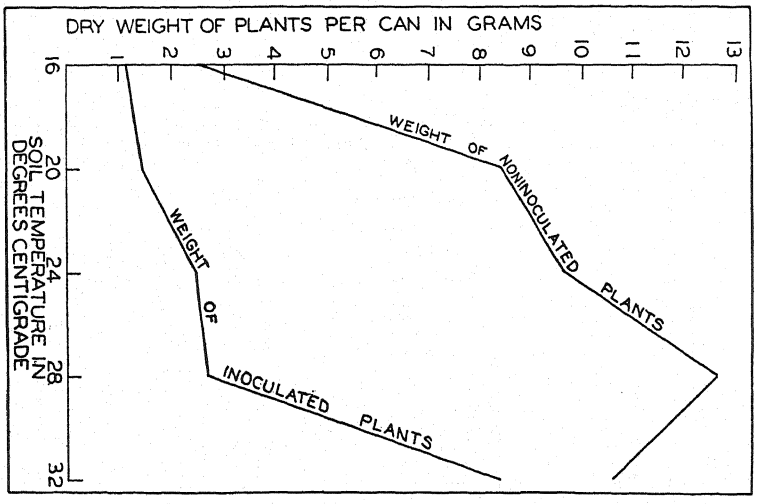


FIG. 2. Influence of soil temperature on weight of noninoculated eggplants and of those inoculated with *Verticillium dahliae* in the 1st tank experiment. Mean air temperature, 19°, soil moisture, 65 per cent of the moisture-holding capacity.

this point are not very consistent, they indicate that the appearance of symptoms may be delayed a few days by very high (28° or above) and by very low (16° or below) soil temperatures. The only appreciable effect of soil moisture on length of incubation period was a slight delay in the appearance of symptoms in plants grown in very moist soil at 28°. This effect was not evident above or below this temperature. It is shown graphically in figure 3.

The results of the first experiment relating to the effect of temperature on growth of inoculated and noninoculated plants in 65 per cent saturated soil are summarized graphically in figure 2. In figure 3 the data from Experiment 2 relating to the effect of 4 different soil moistures at 28° soil

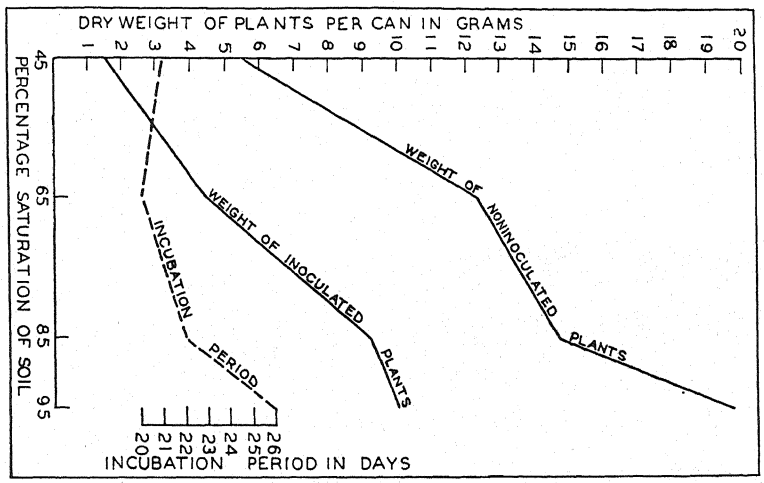


FIG. 3. Influence of soil moisture on weight of noninoculated eggplants and of those inoculated with *Verticillium dahliae* and on the incubation period, in the 2nd tank experiment. Soil temperature, 28°, mean air temperature, 22°.

temperature are plotted. Table 3 contains the weights of all noninoculated plants, and the percentages of reduction in weight of all inoculated plants grown in Experiments 1 and 2. The results of all tank experiments with eggplant will be summarized at the end of the section on eggplant hadromycosis. Thirty degrees was the highest soil temperature at which definite symptoms of eggplant hadromycosis appeared; *Verticillium dahliae* was reisolated from eggplant stems 2 in. above ground level in all 3 inoculated cans at 30° soil temperature in Experiment 2. Photographs were taken of the plants in the cans in Experiment 1 after 5 weeks' growth. The influence of varying soil temperature in soil 85 per cent saturated with water is shown in figure 4 and that of varying soil moisture at a soil-temperature of 28° in figure 5, the inoculated plants being contrasted with the corresponding noninoculated plants in each case.

In order to compare *Verticillium albo-atrum* with *V. dahliae* as to the effect of soil temperature on pathogenicity, the following experiments were conducted. On September 26, 1931, 2 cans with *V. dahliae* (No. 25) and 2 with *V. albo-atrum* (No. 35) were planted with eggplants. One can inoculated with each species was kept at 28° soil temperature and the other at 30°. Two noninoculated cans were kept at each soil temperature. The mean air temperature was 20°. Definite symptoms of wilt appeared about 19 days after planting in each can inoculated with *Verticillium dahliae.* No symptoms appeared in the cans inoculated with *V. albo-atrum* until about the 35th day, and then only in the can at a soil temperature of 28°. Forty days after planting, all the tops were severed from the roots, dried and

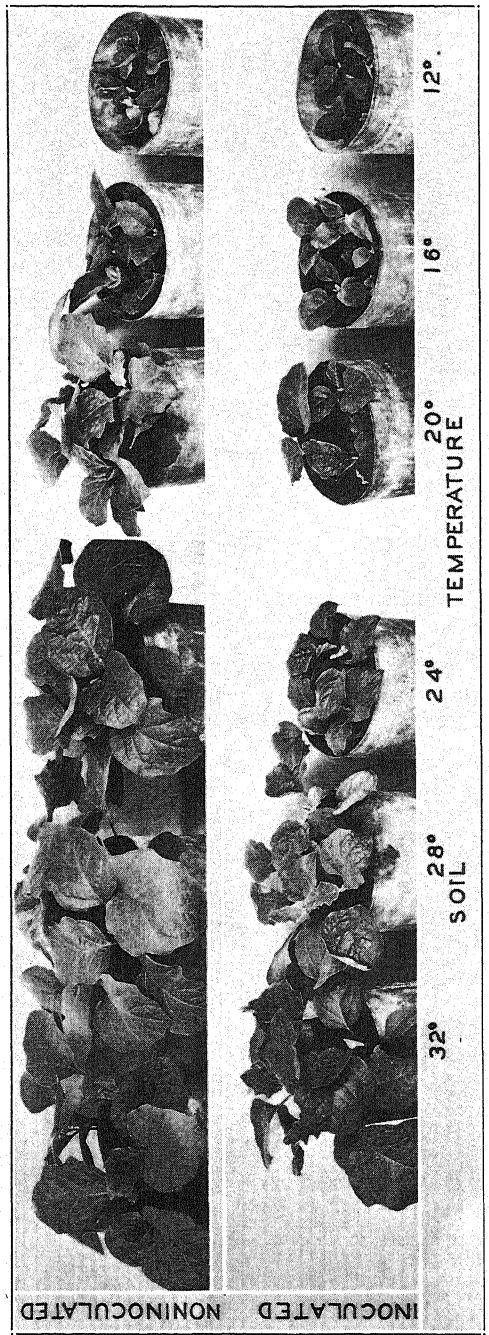


FIG. 4. Influence of soil temperature on growth of noninoculated eggplants and on severity of disease in eggplants inoculated with *Verticillium dahliae*. Soil moisture, 85 per cent of the moisture-holding capacity, mean air temperature, 19°. Five weeks after inoculation.

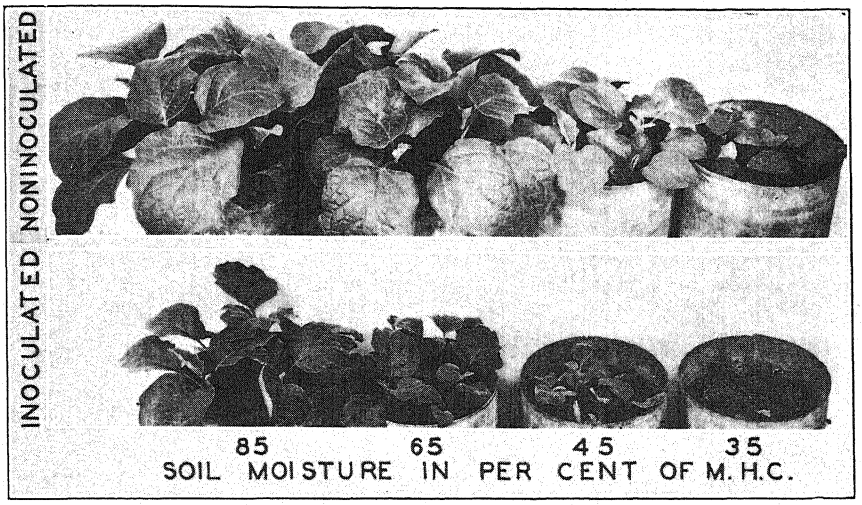


FIG. 5. Influence of soil moisture on growth of noninoculated eggplants and on severity of disease in eggplants inoculated with *Verticillium dahliae* at a soil temperature of 28° and an average air temperature of 19°. Five weeks after inoculation.

weighed. There was about a 30 per cent reduction in the weights of the plants inoculated with *V. dahliae* at 28° and 30°, with marked symptoms of disease in each case. The plants inoculated with *V. albo-atrum* were reduced in weight by 20 per cent at 28° and by 7 per cent at 30°. They displayed slight disease symptoms at 28° and none at 30°.

The same cans of soil were replanted with eggplant seedlings, and similar results again obtained. The can inoculated with *Verticillium albo-atrum* and kept at 30° soil temperature was held for 10 weeks without the appearance of any sign of disease. The experiment was repeated a third time during January and February in a new lot of freshly inoculated soil, the same cultures of Verticillium being used; one other culture of each species, Nos. 14 and 44, was also tried. The results agreed with those of the previous experiments.

A further series was run including 6 other cultures of *Verticillium albo-atrum* at soil temperatures of 28° and 30°, and 4 other cultures of *V. dahliae* at soil temperatures of 30° and 32°. The mean air temperature was about 23°. Of the *V. albo-atrum* cultures at 28°, 4 produced slight symptoms and 2 had no effect; at 30°, none had any effect. All the *V. dahliae* cultures produced definite signs of disease at 30°, but none at 32°. It is therefore concluded that whilst the maximum soil temperature at which *V. albo-atrum* can cause disease in eggplant is usually about 28° or below, it is commonly about 30° in the case of *V. dahliae*. This agrees with the dif-

ference in maximum temperature that has been demonstrated for the growth in culture of the 2 species.

Soil temperature relations at 28° to 31° air temperature. This experiment was begun on April 12, 1931, in a small glass house maintained at a minimum air temperature of 28°. On sunny days the air temperature became very high, often reaching 37° in the middle of the day and raising the mean air temperature over the 10 weeks of the experiment to 31°. *Verticillium dahliae* (No. 25) was used. One inoculated and one noninoculated can were held at each of the following soil temperatures: 22°, 24°, 26°, 28°, 30°, and 32°. The noninoculated eggplants did not make such healthy growth as those in the larger house, where the 1st and 2nd experiments were conducted. Both inoculated and noninoculated plants tended towards a spindling habit of growth with chlorotic areas between the veins of the leaves.

The periods that elapsed before the first appearance of visible symptoms were distinctly longer than in the first 2 experiments, *viz.* 29 days at 22° soil temperature and 31 days at 24°, 26°, and 28° soil temperature. No definite symptoms appeared at 30° or 32°. The lowest 3 or 4 leaves of each inoculated plant at the lowest 4 soil temperatures were wilted and withered by the disease, which progressed slowly during the next 2 or 3 weeks after the first appearance of symptoms.

The eggplants began to outgrow the disease 7 or 8 weeks after planting, presumably owing to the increasingly high air temperatures during the daytime, although the new growth in both control and inoculated plants was of the unhealthy type described above. On June 27, about 10 weeks after planting, the experiment was discontinued. The diseased leaves by that time had dropped off and the remaining foliage was free from any definite symptoms of hadromycosis at all soil temperatures. In the 22° and 24° tanks, the inoculated plants were slightly smaller than the noninoculated plants, but in the others no difference was visible. The inoculated plants at soil temperatures from 22° to 28° showed distinct brown discoloration of the vascular bundles of the stem up to 2 or 3 in. above the soil level, but those at 30° and 32° did not show any such discoloration. *Verticillium dahliae* was reisolated from the stem (about 1 in. above ground level) of the plants at 26° and 28° soil temperatures, but those at 30° failed to give any growth of the fungus on plating.

This experiment indicates that at a high air temperature (averaging about 31°) the maximum soil temperature at which *Verticillium dahliae* may cause disease symptoms in eggplant is somewhat lower than at low air temperatures. Thus, in the experiment just described, the maximum soil temperature at which disease symptoms developed was 28°, as compared with 30° in the 2nd tank experiment. Similar indications were obtained from the 1st tank experiment with tomatoes, described below.

Air temperature relations. In the experiments described above, the air temperature in most cases differed from the soil temperature. For comparison with the results of such experiments, eggplants growing in pots were inoculated through stem wounds above ground level and kept in small glass houses in which the minimum air temperatures were maintained at 17°, 20°, 24°, and 28°. Ten inoculated and 5 noninoculated plants were kept in each house. The plants were cut off at ground level 35 days after inoculation, dried, and weighed. The weights of the inoculated plants averaged about ½ those of the controls at all 4 temperatures. A 2nd experiment was performed in the 28° house only, using 10 controls and 6 plants inoculated with each of the following cultures: 14 and 35 (*Verticillium albo-atrum*), 25 and 44 (*V. dahliae*). The results agreed with those of the 1st experiment, except that the symptoms produced by the 2 cultures of *V. albo-atrum* at 28° were a little slower in appearing and less marked than those due to *V. dahliae.*

Summary. Noninoculated eggplants grew best at soil temperatures of 28° and 30°, the air temperature being 19° to 22°. The higher soil temperature was more favorable at higher soil moistures. A mean air temperature of 31°, often going up to 37° for short periods, proved less favorable than the lower air temperatures. Eggplants in pots grew better at 28° than at lower air temperatures.

Marked disease symptoms appeared, and the growth of eggplants inoculated with *Verticillium dahliae* was reduced 50 to 80 per cent as compared with that of the noninoculated plants, at soil temperatures of 16°, 20°, 24°, 28°, and 30°, the air temperatures being 19° to 22°. The reduction in growth of inoculated plants was most marked at 20°, gradually decreasing at higher temperatures up to 30°. There was in some cases a slight reduction in average weight of inoculated plants at 32°, but this was probably due to experimental error rather than to parasitism by the fungus, as there were no other symptoms and in 2 out of 6 cases the plants in inoculated soil weighed more than did the controls. Apparently the disease was slightly less severe at 16° than at 20°. At an air temperature averaging 31°, no symptoms appeared above 28° soil temperature: the symptoms at 28° and below were much less marked than at the lower air temperatures and disappeared as the mean air temperature became higher towards the close of the experiment. The maximum soil temperature for disease in plants inoculated with *V. albo-atrum* was 28° as compared with 30° for *V. dahliae,* the air temperature being 20° to 23° in each case. Potted plants inoculated with *V. dahliae* through stem wounds above soil level became diseased with approximately equal severity at air temperatures of 17°, 20°, 24°, and 28°.

Increase of soil moisture up to 95 per cent of saturation was accompanied by increased growth of noninoculated eggplants at 24° and 28°

soil temperature; at 30° and 32°, increase of soil moisture up to 85 per cent (the maximum tried at these temperatures) gave increased growth of the noninoculated plants. At 20° and 16°, noninoculated plants in soil 85 per cent saturated grew less than did those in soil 65 per cent saturated.

Soil moisture exerted no appreciable effect on severity of disease except at 28° soil temperature (air temperature 19° to 22°), where the disease was a little later in appearing and less severe in soils 85 and 95 per cent saturated than in those 45 and 65 per cent saturated with water. Probably this was merely an effect of the increased vigor of the host plants under these conditions.

The results as to temperature relations will be compared with data from field observations in the section on "Temperature Relations in the Field."

TOMATO HADROMYCOSIS

Soil temperature relations at 25° to 30° air temperature. The 1st tank experiment with tomato as host was begun on April 12, 1931. The temperatures used were determined by the space left over after requirements had been satisfied for the concurrent eggplant experiments rather than selected with suitability as the first consideration. Larger quantities of inoculum were used than in the experiments with eggplant, as some difficulty had been experienced previously in producing disease symptoms in the tomato. One-hundred-fifty grams were used in each can, made up of equal parts of corn-meal sand inoculated with the following cultures: *Verticillium dahliae* (No. 25) and *V. albo-atrum* (No. 35). One inoculated and one noninoculated can were kept during the whole experiment in the large greenhouse used for the 1st and 2nd eggplant experiments, where the air temperature averaged about 24.5° during this time (23° during the first 5 weeks). The remainder were kept for the first 5 weeks of their growth in the small greenhouse used for the eggplant experiment in which the air temperature averaged about 30° during this time. Here it became so hot during the day that both inoculated and noninoculated plants made very spindly, small-leaved, unhealthy growth, and no definite symptoms of hadromycosis developed. As soon as space became available, these cans were transferred to a larger greenhouse, where the air temperature during the rest of the experiment averaged 26°, and were here kept at lower soil temperatures, as stated in table 4, where the conditions of growth and development of disease in each inoculated can of this experiment are summarized.

It would appear from the results of this experiment that high air temperatures (including temperatures too high for the healthy growth of the host) may hinder the appearance of symptoms in plants growing in soil at temperatures suitable for the optimum development of the fungus.

Soil temperature relations at 20° air temperature. The 2nd tank ex-

TABLE 4.—*Conditions of growth and development of Verticillium hadromycosis in 1st tank experiment with tomato*

Conditions of growth				Approximate incubation period[a]	Final results	
First 5 weeks		Second 5 weeks				
Soil temperature	Average air temperature	Soil temperature	Average air temperature		Browning of vascular bundles	External symptoms
Degrees	*Degrees*	*Degrees*	*Degrees*	*Days*		
22	30	14	26		Slight at base of stem	Indefinite; stunting, drying up of lower leaves
24	"	16	"		"	"
26	"	18	"		"	"
20	23	20	"	18	Marked, visible 9 in. up stem	Marked wilting, yellowing, and withering of lower leaves
28	30	20	"		Slight at base of stem	Indefinite
24	23	24	"	18	Marked	Marked
30	30	24	"		None	Symptoms less marked; gradual drying up of lower leaves
32	"	26	"	No symptoms	None	None

[a] Where no data are given, it was not found possible to assign even an approximate date for the first appearance of symptoms.

periment with tomato was begun on September 26, 1931. The mean air temperature was 20°. Each inoculated can received a total of 150 gm. of inoculum, 50 gm. each of corn-meal-sand medium inoculated with cultures 21 and 25 (*Verticillium dahliae*) and culture 35 (*V. albo-atrum*). Two inoculated and 2 noninoculated cans were kept at each of the following soil temperatures: 20°, 22°, 24°, 26°, 28°, and 30°. It was desired to run tanks at 16° and 18°, but the lowest soil temperature obtainable at the

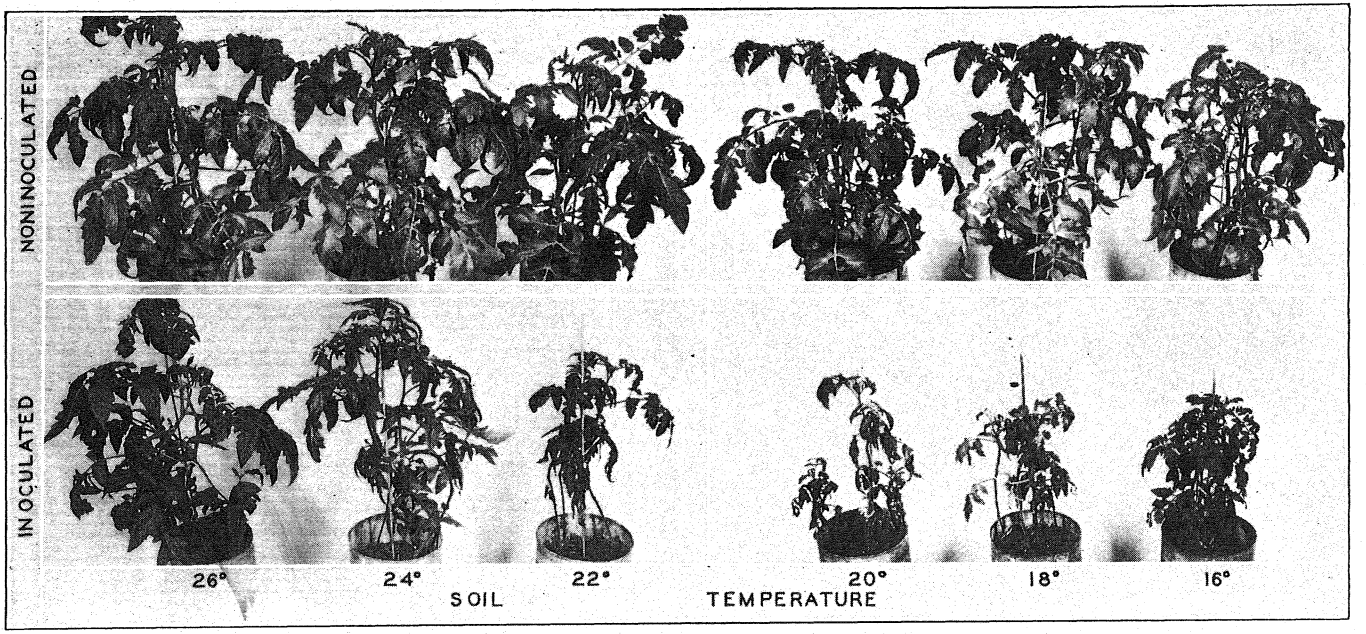


FIG. 6. Influence of soil temperature on growth of noninoculated tomatoes and on severity of disease in tomatoes inoculated with a mixture of *Verticillium albo-atrum* and *V. dahliae*. Mean air temperature, 20°, soil moisture, 65 per cent of the moisture-holding capacity. Six weeks after inoculation.

beginning of the experiment was 19°. Two tanks (with 2 inoculated and 2 noninoculated cans in each) were begun at this temperature; 18° was attained by the end of the 3rd week, and one tank was kept at this temperature. The other reached 16° by the end of the 4th week.

The incubation period in the 2nd experiment varied from 17 days at 20° soil temperature to 20 days at 22° and 25 days at 24°. Severe disease occurred at 22° and below, less severe at 24°, and still less at 26°, at which temperature no definite time could be assigned for the first appearance of symptoms. No symptoms appeared at 28° or 30°. After 6 weeks' growth the plants were photographed (Fig. 6), dried, and weighed. The weights of noninoculated plants and percentages of reduction in weights of inoculated plants are given in table 5. The noninoculated plants grew about equally well at soil temperatures from 20° to 30°.

The 3rd tank experiment with tomato was begun on February 13, 1932. It was a replication of the 2nd except that 3 cans in each tank were inoculated with a pure culture of *Verticillium albo-atrum* (No. 35) and 3 with a

TABLE 5.—*Influence of soil temperature on dry weights in grams of stems and leaves of noninoculated tomato plants from the 2nd and 3rd tank experiments and on reductions in weight of inoculated plants (expressed as percentages of the dry weights of the corresponding noninoculated plants). The results from duplicate cans are averaged in Experiment 2, and those from duplicate noninoculated and triplicate inoculated cans are averaged in Experiment 3. Each weight represents the average total dry weight of stems and leaves per can of 3 plants. Mean air temperature = 20° in each case.*

Soil temperature	Experiment 2. Inoculated with a mixture of *Verticillium albo-atrum* and *V. dahliae*		Experiment 3		
	Weight of noninoculated plants	Percentage of reduction in weight of inoculated plants	Weight of noninoculated plants	Percentage of reduction in weight of plants inoculated with:	
				V. albo-atrum	*V. dahliae*
Degrees					
16	9.7	72	7.1	66	–13[a]
18	10.2	59	–	–	–
20	10.4	77	11.7	68	–5[a]
22	9.1	50	11.4	62	–16[a]
24	10.8	32	12.7	68	–6[a]
26	10.9	38	12.7	53	6
28	10.0	–10[a]	12.1	6	4
30	11.1	0	10.4	–2[a]	–2[a]

[a] Where a minus sign precedes the percentage, it indicates that the weight of the inoculated plants exceeded that of the corresponding noninoculated plants by this percentage, so that the reduction in weight was a minus quantity.

pure culture of *V. dahliae* (No. 25). The remaining 2 cans were noninoculated. Of the 3 cans inoculated with each species of Verticillium at each soil temperature, 2 received 80 gm. and one 160 gm. of corn-meal-sand inoculum. The noninoculated cans each received 80 gm. of sterilized inoculum. The mean air temperature was again 20°.

In the tomato plants inoculated with *Verticillium albo-atrum* in the 3rd experiment, symptoms appeared at all soil temperatures up to and including 26° within 13 to 15 days. During the first 5 weeks' growth, no definite symptoms appeared in any of the plants inoculated with *V. dahliae*. Those inoculated with *V. dahliae* at all soil temperatures and those with *V. albo-atrum* at 28° and 30° were indistinguishable from the noninoculated plants. At the end of 5 weeks' growth the plants were dried and weighed, excepting one plant in each can at 22°, 26°, and 28°. These were kept growing at the same temperatures to see if disease would appear later in the plants not then showing it. *Verticillium albo-atrum* was isolated from the xylem in the base of the stem one in. above ground level in 3 plants at 28°, which showed slight vascular discoloration. This, like similar reisolations made from field-grown inoculated plants (see "Susceptibility of Various Hosts to Verticillium Hadromycosis") proves that the fungus may be present in the lower portions of a tomato plant without appreciably affecting its growth. At this time slight yellowing and wilting of the leaves were beginning to appear at the bases of the plants inoculated with *V. albo-atrum* at 28° and of those inoculated with *V. dahliae* at 28° and below. These plants were otherwise similar to the noninoculated plants (Fig. 7). After



FIG. 7. Pathogenicity and soil temperature relations of *Verticillium dahliae* and *V. albo-atrum* in tomato. A, noninoculated, B, inoculated with *V. dahliae*, C, inoculated with *V. albo-atrum*, all at 26° soil temperature. D, inoculated with *V. albo-atrum* at 28° soil temperature. Mean air temperature, 20°, soil moisture, 65 per cent of the moisture-holding capacity. Five weeks after inoculation.

2 more weeks these symptoms had not become any more marked, though *V. dahliae* was reisolated from the petiole of one of the lower leaves of 2

plants at 26° and 2 at 28°, which showed slight yellowing and tip withering of the lowest 2 or 3 leaves. Thus, *V. dahliae,* though present in the lower parts of the plants, did not significantly affect their growth. Three of the plants inoculated with *V. dahliae* and grown at 22° were kept for a total period of 8 weeks without further symptoms developing than those already described. The weights of plants and percentages of reduction in weight of inoculated plants at the end of 5 weeks' growth are given in table 5. In most cases the plants inoculated with *V. dahliae* weighed slightly more than the noninoculated plants. It is doubtful whether this fact has any significance. As *V. dahliae* alone did not cause appreciable amounts of disease in the 3rd experiment, it is presumed that *V. albo-atrum* was mainly responsible for the disease in both experiments.

The disease occurred with approximately equal severity in tomato plants at soil temperatures ranging from 16° to 22° in both experiments. It was a little slower in appearing and less severe at 24° and 26° in the 2nd experiment; in the 3rd it was less severe at 26° but as severe at 24° as at lower temperatures. There was no significant amount of disease at 28° or 30° in either experiment. There was no difference between the plants receiving 80 gm. of inoculum per can and those receiving 160 gm. in the 3rd experiment. Thus, the maximum soil temperature for the occurrence of Verticillium hadromycosis in tomatoes inoculated with either a mixture of both species or *Verticillium albo-atrum* alone appears to be about 26°, as compared with a maximum of 30° for eggplants inoculated with *V. dahliae* or 28° for eggplants inoculated with *V. albo-atrum*, the air temperature in each case averaging 20°.

Potato Hadromycosis. This experiment was run in the same tanks as were used for the 2nd tomato experiment and was begun at the same time, September 26, 1931. The tubers used were free from vascular discoloration and were surface sterilized. The soil temperatures used were 16° to 19°, 18° to 19° (see description of 2nd tomato experiment for an explanation regarding these two), 20°, 22°, 24°, and 26°. Two inoculated and 2 noninoculated cans were kept at each soil temperature. The inoculum was similar to that used in the 2nd tomato experiment, *i.e.,* a mixture of *Verticillium albo-atrum* and *V. dahliae.* The mean air temperature was 19°.

The growth of both inoculated and noninoculated potato plants was rather weak and spindly, and the leaflets were below the normal size. There was little difference visible between the growth of the noninoculated plants at any of the different soil temperatures used. Signs of disease appeared after very irregular intervals, as is shown in table 6. They were in many cases hard to distinguish from the gradual senescence, yellowing, and withering of the leaves from the lowest up, which took place in non-

TABLE 6.—*Influence of soil temperature on severity of Verticillium hadromycosis, length of incubation period, and relative pathogenicity of V. alboatrum and V. dahliae to the potato*

	Soil temperatures (mean air temperature = 19°)					
	16°	18°	20°	22°	24°	26°
Total weight in grams of tubers from noninoculated plants	99.0	98.0	90.0	97.0	84.5	80.5
Percentage of reduction in weight of tubers from inoculated plants	58	60	34	46	18	10
Estimated percentage of reduction in growth of tops of inoculated plants after 60 days' growth	60	85	70	30	30	20
Approximate incubation periods (in days) for each of the 4 inoculated plants at each soil temperature	33, 50, 55, 60	40, 40, 45, 45,	35, 45, 50, 55	33, 45, 55, 60	45, 50, 55, 60	55, 55, 60, 65
Average incubation period (days)	50	42	46	48	52	59
Results of isolations from inoculated plants	*V. albo-atrum* alone from 3 plants, *V. albo-atrum* + *V. dahliae* from 1 plant	*V. albo-atrum* alone from 2 plants, *V. dahliae* alone from 2 plants	*V. dahliae* alone from 1 plant	*V. albo-atrum* + *V. dahliae* from 1 plant, *V. dahliae* alone from 2 plants	*V. dahliae* alone from 4 plants	*V. dahliae* alone from 4 plants

inoculated potato plants grown in pots in the greenhouse under unfavorable conditions and also in the noninoculated plants of the tank experiment towards the end of their growth. Thus, the times given for the first appearance of symptoms are subject to an error of 5 days or so, due to this uncertainty. The greater length of incubation period in these plants than in the tomatoes or eggplants previously mentioned is accounted for, at least in part, by the fact that in the latter cases seedlings 2 or 3 in. high were planted and in the former case portions of tubers that took much longer to attain an equivalent development of aerial parts. The incubation periods for each of the 4 inoculated plants at each single soil temperature varied considerably (between 33 and 60 days in 2 cases). The disease appeared most rapidly at 18°, taking slightly longer at lower and higher soil temperatures. These results, however, are of doubtful significance.

After about 60 to 65 days the noninoculated plants ceased growth, probably owing to diminishing day length and light intensity; their leaves began to die off slowly from the lowest up, much as did those of the inoculated plants. Three noninoculated plants in which this process began a few days earlier than in the rest were plated, but all proved sterile.

Platings were made during the course of the experiment from the potato plants that developed symptoms of the disease. Though the disease was longer in appearing, it seemed to kill the plants off more rapidly than in the case of tomatoes or eggplants under similar conditions. All the leaves on a plant would be withered and the stem beginning to dry up from the tip downward within about 10 to 14 days of the first signs of disease. Plants were removed for plating while the upper leaves were still alive. Verticillium was isolated from the stem at least 3 in. above ground level, the stem and uppermost leaves being still alive and turgid, from one or more plants at each soil temperature used. The fungus was obtained in every case in which its isolation was attempted. The results of these reisolations are summarized in table 6. Microsclerotia were formed abundantly in stem-tissue cultures from the higher soil temperatures; dark hyphae alone developed in cultures from 3 out of 4 plants grown at 16° soil temperature and from 2 out of 4 plants grown at 18°. Each of 10 single-spore cultures from one 18° plant proved to be *V. albo-atrum,* and each of 10 from one 26° plant proved to be *V. dahliae.* While representing only a few isolations, these results would seem to indicate that *V. albo-atrum* is more actively parasitic on the potato at low soil temperatures and *V. dahliae* at high soil temperatures.

An estimate of the relative amounts of reduction in the aerial growth of inoculated potato plants, expressed as percentages of the amounts of aerial growth made by the noninoculated plants, was made at the end of 60 days' growth and is recorded in table 6. By December 9, after 74 days'

growth, the noninoculated plants were beginning to die; the tubers formed in each can were removed from the soil and weighed. It is thought that the weights thus obtained give the best available expression of the relative total amounts of growth per can, as the aerial parts of diseased plants were removed at various times during the last 2 or 3 weeks of the experiment for reisolation work. The results of the weighings are given in table 6. It is evident from the symptoms developed, the reisolation of Verticillium from the stems, and the reduction in weight of tubers, that there was some disease development at all the soil temperatures used, from 16° to 19° to 26°. The reductions in weight of the tubers agree with the other data in indicating slightly less severity of disease at soil temperatures of 24° and 26° than at 22° and below. Possibly the upper limit is higher than 26°, as no plants were grown above 26°, but the decrease in amount of disease at 26° gives reason to believe that little or no disease development would occur above this temperature. None of the tubers showed any sign of vascular discoloration: 2 tubers from each inoculated can were surface sterilized and pieces cut from them kept in a moist chamber, but no fungal growth appeared. Verticillium had readily been isolated by the writer on previous occasions by the same method from tubers of naturally diseased plants.

It is thought likely that under conditions more favorable to the healthy development of the potato plant and perhaps with a lower concentration of inoculum in the soil, the maximum soil temperature for the development of symptoms might be lower. This hypothesis is suggested by the writer's failure to produce disease symptoms in vigorous field-grown plants, although soil and air temperatures throughout a large part of the growing period were below those at which the disease was produced in the greenhouse. The same applies in the case of the tomato. In other words, it seems likely that the temperature relations of the disease are dependent to some extent upon the vigor of the host plants. Therefore, results gained from soil-temperature-tank investigations do not necessarily hold for field conditions in this case. Further evidence bearing upon this point will be discussed under the next heading.

Temperature Relations in the Field

Under the heading "Pathogenicity of *Verticillium dahliae* and *V. albo-atrum*—Field Experiments" it was pointed out that artificially inoculated eggplants, tomatoes, and potatoes, grown in the field during 1930 and 1931, failed in many cases to develop disease symptoms, though the temperatures prevailing during the greater part of the growing periods were found favorable for disease in plants grown in soil-temperature tanks. Somewhat similar observations have been recorded by Gratz (10). He describes

experiments repeated over several seasons, in which potato tubers infected with Verticillium were collected in Maine; half of these were grown in Maine and half in Florida. The Maine crops contained 25 to 30 per cent of severely diseased plants, while the Florida crops produced 0 to 3 per cent of diseased plants, none of which showed more than very slight symptoms. The temperatures during the first ⅔ of the Florida potato season do not differ significantly from those for the corresponding period in Maine and are well below the maximum for the fungus; Gratz considers that this evidence points to the likelihood of some factor or factors other than temperature being involved in the failure of the disease to appear in Florida. It should be noted, however, that during the last 3rd of the potato-growing season, temperatures are falling in Maine but rising in Florida.

In figure 8, the weekly mean air and soil temperatures during the growing

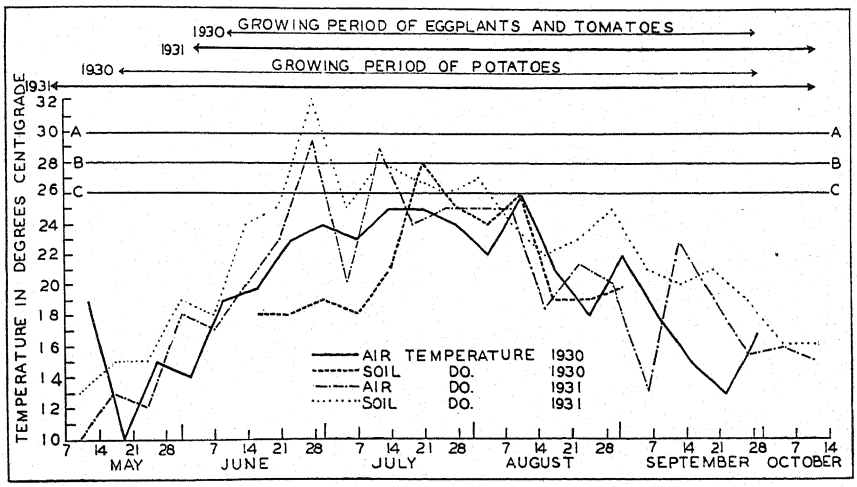


FIG. 8. Weekly mean soil and air temperatures in the field plots during the growing periods of 1930 and 1931, compared with the experimentally derived maxima for the occurrence of Verticillium hadromycosis. The maximum soil temperatures at which disease occurred in eggplants grown in soil-temperature tanks are indicated by the lines A (when air temperature was 22°) and B (when air temperature was 31°). The maximum soil temperature at which disease occurred in tomatoes and potatoes in tanks at 20° air temperature is indicated by the line C. The mean temperature for each week was plotted at a point corresponding to the end of that week.

periods of 1930 and 1931 are plotted for comparison with the highest soil temperatures at which distinct disease symptoms were obtained in the tank experiments. The mean temperatures in figure 8 were derived by averaging the 2-hourly readings of thermographs operated amongst the plants 12 in. above and 4 in. below ground level, with the following exceptions for

periods during which no thermograph was available. The air temperatures up to the middle of June, 1930, and the end of June, 1931, were derived from the United States official weather reports for Madison. The soil temperatures up to the end of June, 1931, were obtained by averaging thermometer readings taken daily at 8:00 A. M. and 7:00 P. M. The values thus obtained are only an approximation of the true weekly means, but it is thought they are more likely to be above than below the true mean temperatures for the following reasons. The bulb of the thermometer was placed 4 in. below soil level in unshaded ground (the roots of the plants would be partly shaded) and the morning reading was not taken till some time after sunrise, so that usually the ground would have warmed up appreciably from its night temperature before the reading was taken. Thus, if the true mean soil temperatures about the roots of the plants differed from the approximate values plotted in figure 8, they were probably lower and, therefore, there would be all the more reason to suppose that disease should have occurred.

In 1930 the weekly mean temperatures throughout the whole of the growing period were well below the maximum for the disease in eggplant, and nearly all the inoculated plants showed moderate symptoms of disease. In 1931 it was only during the last week in June and beginning of July that the weekly mean temperature exceeded the experimental maximum for the disease; during July the soil and air temperatures averaged 27° and 26°, respectively, but throughout the remainder of the growing period they were near or below the optimum for the fungus. Yet, about ⅓ of the 140 eggplants inoculated did not differ appreciably from the noninoculated plants, and most of the remainder showed symptoms that were very mild as compared with those obtained in the tank experiments. It would seem from the results of the tank experiments that more severe disease than this might have been expected to occur in the field. It is not thought that too low temperatures prevented its occurrence, as severe symptoms appeared at a soil temperature of 12° in the tank experiments.

No symptoms appeared in tomato plants except slight vascular discoloration at the base of the stem produced by 6 out of 9 cultures of Verticillium in 1930 and by 1 out of 7 cultures in 1931. The potatoes were unaffected in 1930; in 1931, 2 out of 7 cultures produced slight stunting and vascular discoloration, while the remainder had no effect. In no case were such severe disease symptoms produced in tomatoes or potatoes in the field as were obtained at a constant soil temperature of 26° and an almost constant air temperature of 20° in the tank experiments, using a mixture of *V. dahliae* and *V. albo-atrum* as inoculum. In 1930 the weekly mean soil temperatures for the first 5 weeks after transplanting the tomato seedlings into inoculated soil in the field were 18°, 18°, 19°, 18°, and 21°

(average 19°); the corresponding air temperatures were 20°, 23°, 24°, 23°, and 25° (average 23°). In the first tank experiment with tomatoes, symptoms first became evident within 18 days at soil temperatures of 20° and 24°, the mean air temperature being 23° in each case. The plants were severely diseased within 5 weeks. Similar results were obtained in the 2nd tank experiment at soil and air temperatures of 18° to 20°. In 1931 mean weekly temperatures between the 4th and 9th weeks after planting in the field approximated or exceeded the experimentally determined maximum for the disease in tomato, but there was ample time before and after the periods of heat to permit of the development of disease symptoms, according to the results of the tank experiments. The results of the single inconclusive tank experiment with potatoes also give reason to expect that more than the slight traces of disease produced in the field should have been obtained.

It therefore seems necessary to conclude that tank experiments do not give reliable data as to the maximal temperatures at which Verticillium hadromycosis may occur in the field and that conditions other than temperature are concerned in limiting its severity there. Of course, there was a wide variation between greenhouse and field conditions with regard to soil moisture, light intensity, and probably also humidity; exact data as to these factors are not available. Again, whilst the entire root system of each plant grown in the tanks was maintained at one constant temperature, the soil temperature in the field varied both diurnally and at different depths. Presumably the root systems of the field-grown plants were in a considerably healthier state than those of the tank-grown plants and better able to withstand injury to the finer rootlets, which may conceivably be a factor in injury due to Verticillium. No evidence on this point was obtained. Field-grown plants were naturally larger and more vigorous than those grown in the tank cans; it seems possible that the greater the general vigor of the host plant, the nearer to the optimum for the parasite must the temperature be before marked symptoms of hadromycosis can be produced. Bewley (4), speaking of tomatoes, writes, "There is a distinct relation between hardness of growth and susceptibility to wilt; the harder growing varieties and plants suffering from starvation or a severe check in the young stages being most susceptible to attack." Another possibility is that certain hosts may become liable to attack only when acted upon by predisposing factors at present unknown. Thus, Haenseler (11), speaking of Verticillium hadromycosis of the peach in New Jersey, says "It is very probable that winter injury is a necessary predisposing factor and that the organism is unable to infect a normal tree."

DISCUSSION

The apparent discrepancies between the reports of different workers on this disease are probably due in most cases to two main factors, the variability of the pathogen and the imperfectly understood influence of environment upon the host-parasite relations.

The variability of the pathogen has led to a still unsettled disagreement as to the best mode of taxonomic treatment of the vascular Verticillia. Most cultures described in the literature and 45 out of the 46 representative cultures examined by the writer fall into 2 classes, those that form microsclerotia and those that form dark hyphae without microsclerotia. There are some types that do not readily form any dark mycelium. Many of these will do so on appropriate treatment. A new species, *Verticillium dahliae,* was named by Klebahn to include the microsclerotial type, separating it from the type forming dark hyphae, only. Those who agree with Klebahn apply the designation *V. albo-atrum* to the latter type, alone, but it is often used in general literature to include both types. In addition to *V. dahliae,* several other species of vascular Verticillia have been named, *e.g., V. ovatum* B. and J., *V. tracheiphilum* Curzi, etc., the claims of which to specific rank have been questioned by recent authors. The writer's study deals only with *V. dahliae* and *V. albo-atrum* (using the latter term to include types forming dark hyphae alone).

The classification of these 2 groups as separate species appears to the writer to be both useful and practicable. As pointed out earlier in this paper, the type of dark mycelium formed in culture provides a convenient means of distinction. The usefulness of the distinction depends on the extent to which the 2 groups differ in significant physiological characters, *i.e.,* the need for convenient distinction between them. Probably the most important distinctive physiological characters of these fungi are their pathogenicity and temperature relations. Their maximum temperatures are of particular importance in determining the climatic conditions under which disease may occur. The writer's studies agree with those of previous workers in showing that *Verticillium dahliae* is characterized by a distinctly higher maximum temperature than *V. albo-atrum* for growth in culture. The writer has shown this to apply also to disease production in the case of eggplant. Furthermore, *V. dahliae* is in most cases distinctly less pathogenic to certain hosts, *e.g.,* tomato, potato, and cucumber, than is *V. albo-atrum,* at least when the temperature is equally favorable for the growth of either species of fungus (though certain cultures of *V. dahliae* are more pathogenic to tomato than are certain cultures of *V. albo-atrum*). The work of Berkeley, Madden, and Willison (2) indicates that this difference in pathogenicity is more marked in the case of the cucumber.

The most important environal factor influencing the disease appears to be temperature. The general impression gained from the literature is that little disease is likely to occur above 25°. Soil-temperature-tank studies conducted by the writer showed that the maximum soil temperature for disease was about 30° in the case of eggplants inoculated with *Verticillium dahliae* and about 26° in the case of tomatoes and potatoes inoculated with a mixture of both species, the air temperature being about 20° in each instance. Thus there is a marked difference in maximum temperature for the disease in different hosts. The eggplant is more severely attacked than the tomato or potato at temperatures favorable for disease in any of these hosts. Possibly this accounts for the higher maximum temperature for disease in eggplants.

Field observations indicate that the disease may fail to develop in artificially inoculated field-grown plants when the temperature conditions are such as would permit of severe disease development in soil-temperature tanks. A given strain of *Verticillium dahliae* was found to be less pathogenic to vigorous, rank-growing tomato plants than to stunted plants. These facts and similar observations recorded in the literature are interpreted by the writer as suggesting that the host-parasite relationship involves a continual competition between the host and the parasite. Under this hypothesis any factor that retards the growth of the parasite more than that of the host, such as high temperature, will diminish the severity of disease. This diminishing of disease will be more marked when the host is in a vigorous condition than when the host is weak. It will also be more marked in hosts such as tomato and potato than in eggplant, the former two being less susceptible than the latter under optimum temperature conditions for the fungus. Similarly, any factor increasing the vigor of the host plants more than that of the fungus will lessen the amount of disease. Apparently, the maximum temperature for disease depends not merely on the maximum for growth of the fungus but also on the susceptibility of the host, which, in turn, depends on the species and general state of health of the plants concerned.

In the case of the potato, the somewhat fragmentary evidence available indicates that Verticillium is a comparatively weak parasite on this host. The occurrence of disease seems quite erratic and so dependent on environal conditions that the results of greenhouse experiments are of very limited utility in explaining the occurrence or absence of disease in the field. The environal relations of the disease in this host particularly are in need of further study. In the writer's opinion, the physiology of the fungus in the soil and of the potato plant under field conditions (including the possibility of factors predisposing the host to the disease) should receive careful attention in such a study.

SUMMARY

The taxonomy of the vascular Verticillia is briefly discussed. It is thought that the important differences in pathogenicity and temperature relations between the types differentiated by Klebahn as *Verticillium dahliae* and *V. albo-atrum* warrant their treatment as separate species.

Eight cultures of *V. albo-atrum* and 37 cultures of *V. dahliae* from a number of different hosts and localities were studied. As measured by rate of increase in colony diameter, the average growth rate of *V. albo-atrum* approximately equalled that of *V. dahliae* at 22° but was reduced to about ¼ that of *V. dahliae* at 28°. No growth of *V. albo-atrum* was obtained at 30°, while all cultures of *V. dahliae* tried at this temperature gave varying amounts of growth. The optimum for *V. albo-atrum*, according to the literature, is usually about 23°, but 1 culture of *V. dahliae* gave slightly better growth at 28° than at 22°.

Of 15 recorded hosts inoculated with *V. dahliae* under parallel field conditions, eggplant, okra, cotton, snapdragon, and rose appeared most susceptible; wild sumac, blackberry, and raspberry displayed mild symptoms; potato, tomato, pepper, cucumber, dahlia, lupin, and sweet pea showed no symptoms; though Verticillium was reisolated from potato, tomato, and dahlia.

Of 8 cultures of *V. albo-atrum* tested in the greenhouse for pathogenicity to potted tomatoes, 6 were severely pathogenic and 2 caused little or no disease. Nine out of 37 cultures of *V. dahliae* caused moderate to severe disease symptoms, and the remaining 28 displayed little or no pathogenicity to tomatoes under the conditions of the experiment.

In soil-temperature-tank experiments with eggplant, *V. dahliae* produced marked disease symptoms at soil temperatures of from 12° to 30°, but not at 32°; *V. albo-atrum* caused disease at 28° and below, but not at 30°. The air temperature was between 19° and 23° in each case. At 28° to 31° weekly mean air temperature, no disease was produced by *V. dahliae* above 28° soil temperature, and the symptoms at soil temperatures from 22° to 28° were much milder than in the previous experiments, disappearing towards the end of the experiment as the mean air temperature gradually increased.

Variation of soil moisture between 45 per cent and 95 per cent of the moisture-holding capacity had no appreciable effect on severity of disease in eggplant, except a slight lengthening of the incubation period and reduction in severity of disease with the highest 2 soil moistures at 28° soil temperature, probably due to the greater vigor of the host.

Severe disease in tomatoes was caused by *V. albo-atrum* up to 24° soil temperature, slightly less at 26°, only very slight symptoms at 28° and

none at 30° (air temperature = 20°). The culture of *V. dahliae* used caused very slight or no symptoms and no appreciable reduction in growth at soil temperatures from 16° to 30°, though it was reisolated from plants at 26° and 28° soil temperature. Higher air temperatures hindered the appearance of disease at favorable soil temperatures, as with the eggplant.

In a single tank experiment with potatoes, disease occurred from 16° to 26° soil temperature (air temperature 19°) with a mixture of both species of fungus as inoculum.

The difficulty experienced in obtaining the disease in the field at temperatures below the experimentally derived maxima leads to the belief that some factor or factors other than temperature must have lessened the susceptibility of the field-grown plants to the disease. It is suggested that vigorous plants may be resistant to the disease at temperatures favorable for disease in weaker plants.

DIVISION OF PLANT INDUSTRY, C. S. I. R.,
P. O. BOX 109, CANBERRA CITY,
F. C. T., AUSTRALIA.

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THE PREPARATION AND PROPERTIES OF PENTATHIONIC ACID AND ITS SALTS; ITS TOXICITY TO FUNGI, BACTERIA, AND INSECTS

O. NEAL LIMING[1]

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For the last century, various types of sulphur sprays and dusts have been used as crop protectants with varying degrees of success. The common lime-sulphur sprays and the 300-mesh sulphur dusts are now in universal use as fungicides. Sulphur dusts, in which the particles are very fine and readily oxidized, have proved satisfactory in the control of many plant diseases. But these dusts have apparently insufficient fungicidal properties to control fungous diseases such as Brooks spot and the bitter rot of apple.

Sulphur sprays and dusts have not been used extensively to combat bacterial diseases of plants. This is probably due, at least in part, to the difficulty of applying sprays and dusts to infected parts and at a time when they will not be injurious to the host. Furthermore, it may be that sulphur, in the forms in which it is available, is not sufficiently toxic to satisfactorily control such bacterial diseases as fire blight of apple and bacterial spot of peach.

The use of sulphur as an insecticide has largely been confined to the lime-sulphur sprays. The theory that such sprays are effective because of their causticity has recently been questioned by O'Kane and Conklin (7). Although lime sulphur sprays have given good control of some scale insects, there appears to be, at present, no sulphur insecticide that will satisfactorily control aphid infestations.

The results of laboratory tests made by Young (15) show that pentathionic acid, an oxidation product of sulphur, is toxic to fungi. The more recent tests made by Liming (6) support Young's theory. Lack of control with sulphur of some fungous and bacterial diseases and insect infestations may be due to an inadequate amount of pentathionic acid. It therefore appears that a spray or dust with, or capable of producing, a large amount of the acid would be a good crop protectant against most diseases and insect attacks.

The experimental work presented herein is divided into three main subjects. The first part deals with method of preparation, composition, and properties of three pentathionate salts and pentathionic acid. In the second part are given methods of preparation, composition, and the properties of

[1] Formerly Research Fellow, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station. Resigned March, 1931.

a colloidal sulphur paste, together with some suggestions for its commercial preparation. The third part includes the results of tests to show the effect of pentathionic acid on 9 pathogenic fungi, 3 bacterial plant pathogens, and 3 common species of plant aphids. The results of some preliminary tests of the toxicity of colloidal sulphur also are given.

HISTORICAL REVIEW

Pentathionic acid was discovered by Wackenroder to be the principal acid formed by the reaction of sulphur dioxide and hydrogen sulphide in water. The difficulty encountered in preparing the pure salts led Spring (12) to question the interpretation Wackenroder made of his own results. Spring regarded pentathionic acid as a solution of sulphur in tetrathionic acid. Later, Debus (2) prepared several pentathionate salts and definitely proved the existence of the acid. He gave the structural formula, $H \cdot S_2 \cdot SO_2 \cdot OS \cdot SO_2 \cdot O \cdot H$, to indicate the arrangement of the sulphur atoms in the molecule.

The acid occurs in hydrophilic sulphur sols, plastic sulphur, and ground sulphur. The nature of 2 types of hydrophilic sulphur sols has been investigated by Freundlich (3, pp. 615–625). He found that 1 gm. of sulphur from the Wackenroder solution carried about 0.145 millimol of pentathionic acid; whereas 1 gm. of sulphur, formed when sodium thiosulphate is decomposed by an acid, was found to carry about 0.470 millimol of the acid. Babson (11) found that when thiosulphuric acid is mixed with black soil about 80 per cent of it is changed to pentathionic acid. Jablczynski and Frenkenberg (4) reported that the rapidity of decomposition of thiosulphuric acid is increased by the presence of colloidal sulphur.

Another type, a hydrophobic sulphur sol, prepared by adding an excess of water to an alcoholic solution of sulphur, contains, according to Freundlich (3), no pentathionic acid.

Plastic sulphur has pentathionic acid associated with it. Freundlich (3) believed that the pentathionic acid that is mixed with the plastic or amorphous sulphur greatly inhibits its transformation to crystalline sulphur. He pointed out that the conditions under which amorphous sulphur is prepared also favor the formation of pentathionic acid.

The occurrence of pentathionic acid on ground sulphur was reported by Young and Williams (16). They suggested that the natural oxidation of sulphur gives a continuous yield of the acid but that the amount is too small to bring about hydration of the sulphur particles. The later work of Roach (11) showed that when sulphur is added to black soils about 6 per cent of it is oxidized to pentathionic acid.

Three general methods have been employed in the preparation of pentathionic acid. The sodium thiosulphate-hydrochloric acid method is

probably the most satisfactory one of the three. By this method it is possible to prepare a solution containing principally pentathionic acid and its sodium salt. Under favorable conditions and in the presence of a small quantity of arsenious acid, the complete conversion of thiosulphate into pentathionate is theoretically possible. Bassett and Durrant (1) give the following equations to represent these reactions.

$$H_2S_2O_3 + H_3AsO_3 \longrightarrow H_2SO_3 + H_3AsO_3S$$
$$H_2S_3O_6 + H_3AsO_3S \longrightarrow H_2S_4O_6 + H_3AsO_3$$
$$H_2S_4O_6 + H_3AsO_3S \longrightarrow H_2S_5O_6 + H_3AsO_3$$

The sulphur dioxide-hydrogen sulphide method consists of passing hydrogen sulphide into a concentrated solution of sulphurous acid. According to Debus (2), the resulting solution is a mixture of the polythionic acids. Raschig (8) and Riesenfeld and Feld (10) have given the following equations to explain the reaction.

$2SO_2 + H_2S \longrightarrow H_2S_3O_4$ Hypothetical acid
$H_2S_3O_4 + SO_2 \longrightarrow H_2S_4O_6$ Oxidation by sulphur dioxide
$H_2S_3O_4 + 6SO_2 \longrightarrow H_2S_3O_6$ Oxidation by sulphur dioxide
$5H_2S_3O_4 \longrightarrow 3H_2S_5O_6 + 2H_2O$ Polymerization

The amount of acid formed per unit volume by this method is so small that considerable difficulty is encountered in reducing the solution to the concentration necessary for crystallization of a salt in presence of a base.

When sulphur monochloride in aqueous solution is added in small quantities to a pasty mixture of barium thiosulphate and water, a solution is obtained that exhibits all of the properties of pentathionic acid. Spring (12) believed that the product of these reactions is a mixture of polythionic acids.

Young (15) tested the effect of hydrophilic sulphur on 8 pathogenic fungi and found it toxic only between pH 4.2 and 5.4. In his experiments, the colloidal sulphur was coagulated and centrifuged from the "mother liquid" and then peptized in distilled water. Tisdale (13) tested the toxicity of sulphur precipitated from lime sulphur solution, along with the hydrophilic sulphur sols and found them toxic to 8 pathogenic fungi. He reported that aeration and slight desiccation do not destroy the toxic factor.

The results of toxicity tests made by Liming (6) show that, of the acids of sulphur, the polythionic acids are the most toxic to spores of *Sclerotinia cinerea*. Pentathionic acid prepared from the barium salt was found to be toxic in varying degrees to several pathogenic fungi. Filtrates from ground sulphur that contained pentathionic acid were likewise found to be toxic to spores of *S. cinerea*.

THE PREPARATION AND PROPERTIES OF PENTATHIONATE SALTS AND OF PENTATHIONIC ACID

For detailed and exact studies of the effect of pentathionic acid on biological materials, acid solutions from pentathionate salts were used. Such solutions, the exact composition of which is known, are, for laboratory test, highly preferable to commercial materials. Owing to the constant composition of the recrystallized salts, it is possible to prepare at all times solutions that are alike with reference to the composition of the acid in them. The results of toxicity tests in which such acid solutions are used are more reliable, and the results of various tests can be satisfactorily compared with each other.

PERPARATION OF SODIUM, BARIUM, AND POTASSIUM PENTATIONATES

Raschig's method, which employs the reaction between a thiosulphate and an acid in the presence of arsenic, was used in this investigation. The procedure was modified in some respects in an effort to increase the yield of pentathionic acid. One hundred and twenty-five grams of sodium thiosulphate were dissolved in 150 cc. of water, and then to this 2.5 gm. of arsenous oxide, dissolved in a small amount of sodium hydroxide, were added. The temperature of the solution was reduced to $-10°$ C. in a salt-ice bath. Then, 200 cc. of concentrated hydrochloric acid, likewise cooled to $-10°$ C., were added, 5 cc. at a time. The solution was vigorously stirred and kept at the low temperature during the procedure. The resultant precipitate of sulphur, arsenous sulphide, sodium chloride, and sodium sulphate was removed by filtartion.

The filtrate was placed in a porcelain evaporating dish and set before an electric fan. After about 4 hours fanning, the solution was again filtered to remove the precipitate. This procedure was repeated three times, at the conclusion of which the solution had been reduced in volume from 350 cc. to 100 cc., and all the free sulphur and sodium chloride had been removed. It was necessary, during this procedure, to keep the temperature of the solution below 35° C. to inhibit the separation of sulphur.

About 100 cc. of absolute alcohol were added to crystallize the sodium pentathionate. It was observed that if the alcohol was added with sufficient care a layer formed on top of the solution and crystallization took place readily. The next day the sodium pentathionate was removed, thoroughly washed with absolute alcohol, and dried at room temperature.

The filtrate was freed of alcohol by evaporation and then divided into two equal parts. To one part was added 50 cc. of a saturated solution of barium acetate containing 3 per cent acetic acid. A precipitate of barium sulphate was formed, thus removing from solution all of the sulphuric

acid formed by the thiosulphate-acid reaction. The precipitate was removed and saved for quantitative determination. The solution was concentrated by fanning until its volume was again about 50 cc.; to this was added an equal volume of absolute alcohol. The same method of crystallization, filtration, and washing was followed here as described above for the sodium salt.

The other part was treated with just enough barium hydroxide to remove the sulphuric acid. Then, 50 cc. of a 20 per cent solution of potassium acetate plus 3 per cent acetic acid were added. From this point on the procedure was the same as that followed in the preparation of the barium pentathionate.

The filtrates from the two parts were combined and the amount of pentathionic acid that was left in solution was determined. The amounts of free sulphur and various compounds from two such mixtures are given in table 1.

TABLE 1.—*Quantities of free sulphur and sulphur compounds produced by the reaction of 250 gm. of sodium thiosulphate with hydrochloric acid. (Combination of products from two 125 gm. mixtures as recorded in text)*

Sulphur compounds	Compounds	Sulphur	Total S. in the compounds
	gm.	*gm.*	*Per ct.*
As_2S_3	3.07	1.20	1.9
$BaSO_4$	6.13	.84	1.3
Na_2SO_4	trace	.....	.....
$Na_2S_5O_6 \cdot 4H_2O$	31.90	13.81	21.2
$BaS_5O_6 \cdot 3\frac{1}{2}H_2O$	19.78	7.00	10.8
$K_2S_5O_6 \cdot 1\frac{1}{2}H_2O$	21.31	9.45	14.6
$H_2S_5O_6$	12.12	7.52	11.6
Total	.....	39.82	61.4
Free sulphur (theoretical)	24.76	24.76	38.6
$Na_2S_2O_3 \cdot 5H_2O$	250.00	64.58	.....

The results show that about 60 per cent of the sulphur in the thiosulphate molecule formed pentathionic acid. The difference in the amounts of the various pentathionate salts is not significant, as the amount of the base added and the time were probably determining factors.

The Physical Properties of Pentathionate Salts

All three of the pentathionate salts are stable when kept dry at room temperature. Samples of the salts were kept in moisture-proof containers

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PERPARATION OF SODIUM, BARIUM, AND POTASSIUM PENTATIONATES

Raschig's method, which employs the reaction between a thiosulphate and an acid in the presence of arsenic, was used in this investigation. The procedure was modified in some respects in an effort to increase the yield of pentathionic acid. One hundred and twenty-five grams of sodium thiosulphate were dissolved in 150 cc. of water, and then to this 2.5 gm. of arsenous oxide, dissolved in a small amount of sodium hydroxide, were added. The temperature of the solution was reduced to $-10°$ C. in a salt-ice bath. Then, 200 cc. of concentrated hydrochloric acid, likewise cooled to $-10°$ C., were added, 5 cc. at a time. The solution was vigorously stirred and kept at the low temperature during the procedure. The resultant precipitate of sulphur, arsenous sulphide, sodium chloride, and sodium sulphate was removed by filtartion.

The filtrate was placed in a porcelain evaporating dish and set before an electric fan. After about 4 hours fanning, the solution was again filtered to remove the precipitate. This procedure was repeated three times, at the conclusion of which the solution had been reduced in volume from 350 cc. to 100 cc., and all the free sulphur and sodium chloride had been removed. It was necessary, during this procedure, to keep the temperature of the solution below 35° C. to inhibit the separation of sulphur.

About 100 cc. of absolute alcohol were added to crystallize the sodium pentathionate. It was observed that if the alcohol was added with sufficient care a layer formed on top of the solution and crystallization took place readily. The next day the sodium pentathionate was removed, thoroughly washed with absolute alcohol, and dried at room temperature.

The filtrate was freed of alcohol by evaporation and then divided into two equal parts. To one part was added 50 cc. of a saturated solution of barium acetate containing 3 per cent acetic acid. A precipitate of barium sulphate was formed, thus removing from solution all of the sulphuric

acid formed by the thiosulphate-acid reaction. The precipitate was removed and saved for quantitative determination. The solution was concentrated by fanning until its volume was again about 50 cc.; to this was added an equal volume of absolute alcohol. The same method of crystallization, filtration, and washing was followed here as described above for the sodium salt.

The other part was treated with just enough barium hydroxide to remove the sulphuric acid. Then, 50 cc. of a 20 per cent solution of potassium acetate plus 3 per cent acetic acid were added. From this point on the procedure was the same as that followed in the preparation of the barium pentathionate.

The filtrates from the two parts were combined and the amount of pentathionic acid that was left in solution was determined. The amounts of free sulphur and various compounds from two such mixtures are given in table 1.

TABLE 1.—*Quantities of free sulphur and sulphur compounds produced by the reaction of 250 gm. of sodium thiosulphate with hydrochloric acid. (Combination of products from two 125 gm. mixtures as recorded in text)*

Sulphur compounds	Compounds	Sulphur	Total S. in the compounds
	gm.	*gm.*	*Per ct.*
As_2S_3	3.07	1.20	1.9
$BaSO_4$	6.13	.84	1.3
Na_2SO_4	trace	.....	.....
$Na_2S_5O_6 \cdot 4H_2O$	31.90	13.81	21.2
$BaS_5O_6 \cdot 3\frac{1}{2}H_2O$	19.78	7.00	10.8
$K_2S_5O_6 \cdot 1\frac{1}{2}H_2O$	21.31	9.45	14.6
$H_2S_5O_6$	12.12	7.52	11.6
Total	.....	39.82	61.4
Free sulphur (theoretical)	24.76	24.76	38.6
$Na_2S_2O_3 \cdot 5H_2O$	250.00	64.58	.....

The results show that about 60 per cent of the sulphur in the thiosulphate molecule formed pentathionic acid. The difference in the amounts of the various pentathionate salts is not significant, as the amount of the base added and the time were probably determining factors.

The Physical Properties of Pentathionate Salts

All three of the pentathionate salts are stable when kept dry at room temperature. Samples of the salts were kept in moisture-proof containers

for nearly a year, and no important decomposition occurred. A sample of sodium pentathionate, kept on a watch crystal in a desk in the laboratory for 6 months, showed no signs of decomposition.

Samples kept in a moist atmosphere, however, began to break down almost immediately, gave off sulphur dioxide, and became yellow because of formation of free sulphur. Likewise, samples that were not washed thoroughly with absolute alcohol and completely dried were unstable.

Samples of the 3 pentathionate salts were examined by V. H. Morris, Biochemist, Department of Agronomy. The descriptions and drawings of the crystals, and their optical properties, as contributed by him, are given in the following paragraphs.

The optical and crystallographic properties of the potassium and barium salts of pentathionic acid have already been described by C. W. Mason (14, pp. 398–400). The samples of the same salts, prepared as described above, were found to have identical properties.

Sodium pentathionate. The crystals obtained were lamellar in habit, very thin plates that tended to adhere in groups and clusters. They belong to the orthorhombic system. The plates usually are considerably elongated in the direction designated as *c* and flattened parallel to *c* and *b* (Fig. 1, A).

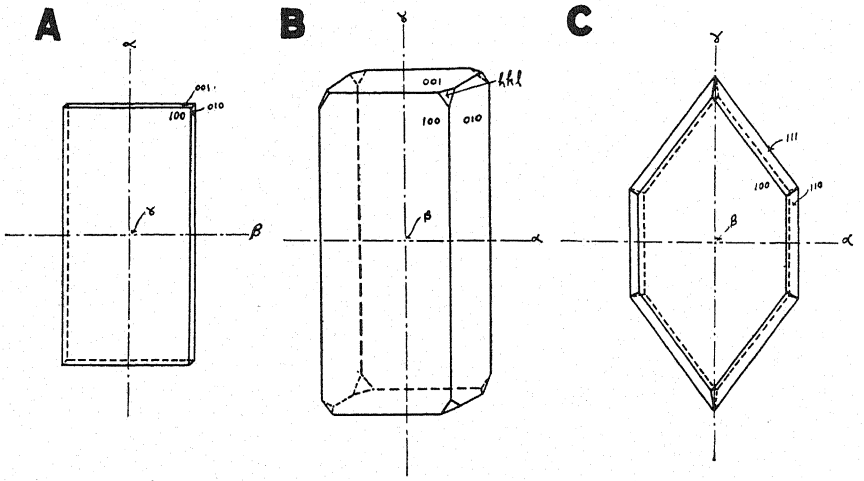


FIG. 1. Crystal habit and optical orientation of: A. Sodium pentathionate. B. Barium pentathionate. C. Potassium pentathionate.

The plates exhibit parallel extinction in all directions. The axial plane is 010; the obtuse bisectrix is *a* and the optic normal *b*. The sign of elongation is negative, as is also the optical character. The refraction indices by the immersion method are: $\alpha = 1.551$; $\beta = 1.605$; and $\gamma = 1.610$.

The above plates, dissolved in water and recrystallized by the addition of water, were obtained as imperfect, tapering, rectangular tablets. The thicker crystals do not exhibit complete extinction, and normal interference figures could not be obtained. The refraction indices of α and β are the same as those of the plates described above and are in the same orientation. These crystals appear to be aggregates of plates similar to those above but adhering together with a definite orientation.

Barium pentathionate. The crystals are most commonly observed as rectangular tablets belonging to the orthorhombic system. They usually are elongated in the direction taken as *c* and the corners are often truncated by small bipyramids (Fig. 1, B).

The tablets exhibit parallel extinction in all directions, and biaxial interference figures may easily be obtained. The axial plane is 100; the optic normal is *a,* and *c* is the acute bisectric. The sign of elongation is positive, as is also the optical character of the crystals. The refraction indices by the immersion method are $\alpha = 1.620$; $\beta = 1.640$; and $\gamma = 1.670$.

Potassium pentathionate. The crystals belong to the orthorhombic system, and the most common crystal habit is illustrated in figure 1, C. If the crystallographic axes are located so that the flattening is parallel to *b* and *c,* the largest faces may be designated 100, and these are beveled by 110 and 111. The crystals are thus hexagonal, with terminal angles of about 74°.

With the crystals oriented as illustrated, the axial plane is 100; the acute bisectric is *c* and the optic normal is *a.* The sign of elongation and the optical character of the crystals are positive. The refraction indices by the immersion method are: $\alpha = 1.570$; $\beta = 1.630$; and $\gamma = 1.658$.

Quantitative Analysis of Pentathionate Salts

The amounts of sulphur and bases in sodium, barium, and potassium pentathionates were determined. In all cases the sulphur was oxidized to a sulphate and measured gravimetrically as barium sulphate. In the case of the barium pentathionate, the barium was precipitated from an aqueous solution as a sulphate and weighed. The amount of base in each of the other salts was determined by oxidizing the sulphur to a sulphate, evaporating the solution to a constant weight, and measuring the residue as sodium or potassium sulphate, as the case might be. Only a brief outline of the procedure for each of the salts is given here.

Sodium pentathionate. A small amount of the salt (0.3826 gm.) was dissolved in 10 cc. of distilled water. Five cc. of a 10 per cent bromine solution were carefully added. The solution was allowed to stand 15 minutes, and then 10 cc. of nitric acid were added. It was warmed until the reaction ceased and the bromine was volatilized. The volume was made up

to 30 cc. and then divided into 2 equal parts. One part was evaporated to dryness, treated with 10 cc. of 10 per cent hydrochloric acid, and dried again to drive off the nitric acid. After the solution was diluted to about 200 cc., 5 cc. of 10 per cent hydrochloric acid, and then 50 cc. of 0.5 N. barium chloride were added. The resultant precipitate, barium sulphate, was collected on a weighed Gooch crucible, ignited, cooled in a desiccator, and weighed as barium sulphate.

The precipitate weighed 0.5927 gm. and therefore contained 0.0813 gm. of sulphur. Theoretically, such a sample should contain 0.0828 gm. The discrepancy of 0.0015 gm. was probably caused by an impurity such as sodium chloride.

The second portion was treated in the same manner as the first, except that sulphuric instead of hydrochloric acid was added. Then the solution was evaporated to dryness and constant weight in a weighted platinum dish. From the amount of the sodium sulphate (0.0774 gm.) thus formed, the amount of sodium was found to be 0.0241 gm. This amount is 0.0004 gm. more than the amount theoretically present in 0.1913 gm. of sodium pentathionate.

Barium pentathionate. By the method described above, the amount of sulphur in 0.3060 gm. of barium pentathionate was determined. From this sample 0.7842 gm. of barium sulphate was formed. The results indicate that the sample contained 0.1075 gm. of sulphur, an amount that is 0.0007 gm. less than the theoretical amount.

For the determination of the barium, 0.3060 gm. of the salt was dissolved in 10 cc. of water. To this solution 10 cc. of 5 per cent sulphuric

TABLE 2.—*Results of quantitative analyses of sodium, barium, and potassium pentathionates*

Pentathionate salts and molecular weights	Experimental results and calculations	Theoretical calculations
$Na_2S_5O_6 \cdot 4H_2O$ 370.30	× : .5927: : 32: 233.43	× : .1913: : 160.3: 370.3
	× equals 0.0813 gm. S.	× equals 0.0828 gm. S.
	× : .0744: : 46: 142.06	× : .1913: : 46: 370.3
	× equals 0.0241 gm. Na.	× equals 0.0237 gm. Na.
$K_2S_5O_6 \cdot 1\frac{1}{2}H_2O$ 361.53	× : .9264: : 32: 233.43	× : .2909: : 160.3: 361.53
	× equals 0.1270 gm. S.	× equals 0.1290 gm. S.
	× : .1413: : 78.2: 174.26	× : .2909: : 78.2: 361.53
	× equals 0.0634 gm. K.	× equals 0.0629 gm. K.
$BaS_5O_6 \cdot 3\frac{1}{2}H_2O$ 453.17	× : .7842: : 32: 233.43	× : .3060: : 160.3: 453.17
	× equals 0.1075 gm. S.	× equals 0.1082 gm. S.
	× : .1582: : 137.37: 233.43	× : .3060: : 137.37: 453.17
	× equals 0.0931 gm. Ba.	× equals 0.0928 gm. Ba.

acid were added. From the weight of the precipitated barium sulphate (0.1582 gm.), it was calculated that 0.0931 gm. of barium was present. The difference between this and the theoretical value (0.09 gm.) is not significant.

Potassium pentathionate. A 0.5818-gm. sample of the salt was used in the determination of the amounts of sulphur and potassium by the same methods employed in the analysis of the sodium salt. Only 0.9262 gm. of barium sulphate, carrying 0.1270 gm. of sulphur, was obtained from 0.2909 gm. of potassium pentathionate.

From the other half of the sample, 0.1413 gm. of potassium sulphate was obtained. This amount of potassium sulphate contains 0.0634 gm. of potassium, which is 0.0005 gm. more than the amount actually present in 0.2909 gm. of the pure salt.

The results of the 6 quantitative analyses and the formulae used in the calculations are given in table 2.

The Preparation and Physical Properties of Pentathionic Acid

A brief outline of 3 general methods for the preparation of pentathionic acid has been given in the historical review of this paper. One of these methods, the thiosulphate-acid reaction, has been described in detail in the section dealing with the preparation of the 3 pentathionate salts.

A concentrated solution of pentathionic acid, comparable in strength to that from which the pentathionate salts were prepared, slowly decomposed on long standing in the laboratory. Free sulphur and sulphur dioxide were the principal decomposition products. After standing 15 days, sufficient sulphur was formed and held in suspension to give the solution a yellow color. Pentathionic acid, which was prepared in dilute solutions from the barium salt, was quite stable. Only a trace of free sulphur and sulphur dioxide was found in the solution after standing 6 months.

The Analyses of Polythionic Acids

For quantitative analyses, a stock solution containing only the polythionic acids was prepared. For this solution, 1.5 gm. of barium pentathionate were dissolved in 16.4 cc. of water; to this were added 33.6 cc. of .2 N. sulphuric acid. The resultant precipitate of barium sulphate was removed by filtration. Qualitative tests showed that the solution contained principally pentathionic, some tetra- and trithionic, but no sulphuric, acids. The amount of each was determined in the presence of each other by 3 volumetric titrations.

The total amount of polythionates was found by measuring the acid formed from the double decomposition of mercuric chloride. A 10-cc. por-

tion of the stock solution was pipetted into 10 cc. of a 10 per cent solution of mercuric chloride. This was well mixed, allowed to stand 10 minutes, diluted to 50 cc., and heated to boiling. After cooling, 25 cc. of 10 per cent ammonium chloride were added to prevent the separation of sulphur in the subsequent titration. The solution was then titrated with .1 N. sodium hydroxide, methyl orange being used as an indicator. For this sample, 26.9 cc. of alkali were required to neutralize the acid formed by the mercuric chloride reaction.

The polythionates react with mercuric chloride, according to Kurtenacker and Bittner (5), as follows:

$$2\,S_3O_6 + 3\,HgCl_2 \rightarrow Hg_3S_2Cl_2 + 4\,Cl + 8\,H$$
$$2\,S_4O_6 + 3\,HgCl_2 \rightarrow Hg_3S_2Cl_2 + 4\,Cl + 8\,H + 2\,S$$
$$2\,S_5O_6 + 3\,HgCl_2 \rightarrow Hg_3S_2Cl_2 + 4\,Cl + 8\,H + 4\,S$$

The equations show that for every 6 mol. of polythionates there were 24 mol. of acid released. Then it follows that the total mols of the polythionates, $S_3O_6 + S_4O_6 + S_5O_6 = a/4$, in which "a" represents the mols of sodium hydroxide used in the titration. The results show that 0.3 gm. (the amount contained in 10 cc. of the stock solution) of the barium salt contains 0.6725 millimol of polythionates.

The total amount of tetra- and pentathionates was determined by the action of a sulphite on the acids. Another 10-cc. portion of the stock solution was treated with 10 cc. of a 10 per cent solution of sodium sulphite and then allowed to stand 10 minutes. To this were added 5 cc. of formaldehyde and 10 cc. of 10 per cent acetic acid. The solution was then titrated with .1 N. iodine, starch being used as an indicator. This solution required 13.1 cc. of .1 N. iodine to neutralize the thiosulphate formed by the reaction.

According to Raschig (8), the thiosulphate was formed by the following reactions:

$$S_4O_6 + SO_3 \rightarrow S_3O_6 + S_2O_3$$
$$S_5O_6 + 2\,SO_3 \rightarrow S_3O_6 + 2\,S_2O_3$$

The trithionate does not react with the sulphite. The equations show that one mol of iodine was required for each equivalent of tetrathionate, whereas two were required for each pentathionate. These conditions can be represented by $S_4O_6 + 2\,S_5O_6 = b$, where "b" is equivalent to the amount of normal iodine used. The results show that, in this case, "b" represents 1.31 millimol.

By another reaction the total amount of the tetra- and pentathionates was determined. But, unlike the sulphite reaction, each of the 2 higher thionates yields the same amount of thiosulphate. Ten cubic centimeters of the stock solution were diluted to 100 cc., and then 5 cc. of a 10 per cent

solution of potassium cyanide were added. After five minutes' standing, the solution was treated with 5 cc. of formaldehyde and 5 cc. of 10 per cent sulphuric acid. The solution was titrated immediately with .1 N. iodine, 6.65 cc. being required to neutralize the thiosulphate.

Kurtenacker and Bittner (5) state that the following reactions take place:

$$S_3O_6 + 3\ CN \rightarrow SO_3 + CNS$$
$$S_4O_6 + 3\ CN \rightarrow S_2O_3 + CNS$$
$$S_5O_6 + 4\ CN \rightarrow S_2O_3 + 2\ CNS$$

The first equation shows that the trithionate produced a sulphite. Due, however, to the presence of formaldehyde, this sulphite did not enter into the iodine reaction. By use of the equation, $S_4O_6 + S_5O_6 = c$, where "c" is the amount of normal iodine consumed, it is shown that there was 0.665 millimol of tetra- and pentathionates.

From the results of the 3 titrations, the amount of each of the 3 polythionates was calculated as follows:

$$a = S_3O_6 + S_4O_6 + S_5O_6 = 0.6725 \text{ millimol}$$
$$b = S_4O_6 + 2\,S_5O_6 = 1.3100 \text{ millimol}$$
$$c = S_4O_6 + S_5O_6 = 0.6650 \text{ millimol}$$

Therefore,

$$S_3O_6 = 0.6725 - 0.6650 = 0.0075 \text{ millimol}$$
$$S_4O_6 = 2(0.665) - 1.310 = 0.0200 \text{ millimol}$$
$$S_5O_6 = 1.310 - 0.665 = 0.6450 \text{ millimol.}$$

From the amount of pentathionic acid found in a 0.3 gm. sample, it was calculated that the salt contained 0.2923 gm. (0.645 times 453.17) of barium pentathionate ($BaS_5O_6 \cdot 3\frac{1}{2}H_2O$). Therefore, the salt was about 97.4 per cent barium pentathionate. Further, the total amount of sulphur accounted for in the 0.6725 millimol of the polythionic acids was 0.1065 gm. By gravimetric methods, 0.306 gm. of the salt was found to contain 0.1075 gm. of sulphur. From this, it was calculated that 0.3 gm. contained 0.1054 gm. of sulphur. The discrepancy of 0.0011 gm. is attributed to experimental error.

HYDROPHILIC COLLOIDAL SULPHUR

Hydrophilic colloidal sulphur has never been used extensively as a fungicide or an insecticide. Laboratory tests have shown that the spray is effective, but the cost of sulphur in this form always has been a limiting factor in its use. Usually, the colloidal sulphur has been coagulated, removed from the "mother liquid," and then dried. Tisdale (13) found that aged colloidal sulphur could not be easily suspended in water, and furthermore, he found that most of its toxicity to fungi was lost.

A sulphur paste was prepared in this laboratory by a method in which the coagulating and centrifuging were omitted and the colloidal properties and the toxicity of the sulphur were not materially reduced. A hydrophilic colloidal suspension, containing sulphur and pentathionic acid, was reduced by evaporation to a thick paste. The pentathionic acid, which stabilizes the colloidal suspension and which is toxic to fungi, was retained in the paste.

PREPARATION OF COLLOIDAL SULPHUR

The Wackenroder method, which employs the reaction of sulphur dioxide and hydrogen sulphide in water, was modified to some extent and used in the preparation of hydrophilic colloidal sulphur.

The amounts, 40 l. of sulphur dioxide, 20 l. of hydrogen sulphide, and 1 l. of water, were chosen arbitrarily for the experiment. One half of the sulphur dioxide was introduced into the water. The temperature was lowered to 10° C. and held near this point during the entire procedure. Then, 5 l. of gas, consisting of equal parts, by volume, of sulphur dioxide and hydrogen sulphide, were bubbled into the sulphurous acid at the rate of 10 l. per hour. The gas was then shut off, and the suspension was allowed to stand 2 hours. This procedure was repeated 8 times.

All of the gas that escaped from the reaction chamber during the process was collected over glycerine and later returned to the suspension. After all of the gas was consumed, the suspension was allowed to stand overnight. During this time some sulphur dioxide was formed. A large quantity of sulphur settled out, but the suspension remained sulphur yellow. More sulphur dioxide was formed when the suspension was removed from the ice bath. This gas was driven off by aeration and collected in an iodine solution for quantitative determination.

The suspension was transferred to a weighed porcelain evaporating dish and placed in front of an electric fan. During the evaporation process the temperature never exceeded 35° C. By this method the water was driven off until a thick paste, weighing 125 gm. was formed. At this time a faint odor of sulphur dioxide was noticeable. However, the amount was so small that it was not considered in the subsequent quantitative analyses.

Qualitative tests showed that the paste contained sulphur, and sulphurous (and possibly thiosulphuric), sulphuric, pentathionic, and traces of other polythionic acids. The amounts of the sulphur and the various acids were determined by the method described earlier in this paper.

The results of the quantitative analyses are tabulated in table 3.

The results from 3 experiments show that such a paste, on an average, is about 68 per cent sulphur, about 69 per cent of the sulphur being in a free state and 31 per cent, combined in ions of the various acids.

TABLE 3.—*Amounts of sulphur and sulphurous (and possibly thiosulphuric) sulphuric, and polythionic acids in 125 gm. of colloidal sulphur paste prepared from about 40 l. of sulphur dioxide, 20 l. of hydrogen sulphide, and 1 l. of water*

Test	Grams of sulphur in source materials			Grams of sulphur in the various constituents of the paste			
	SO_2	H_2S	Total S.	H_2SO_3	H_2SO_4	$H_2S_5O_6$	Free S.
1	57.1	28.5	85.6	0.05	0.13	26.06	59.21
2	56.7	28.5	85.2	0.03	0.08	26.02	58.74
3	58.2	28.5	86.7	0.07	0.17	26.49	59.19
Total	172.0	85.5	257.5	0.15	0.38	78.57	177.14
Percentage of sulphur				0.06	0.15	30.5	68.8

Stated in a more general way, the paste is composed of free sulphur (47 per cent), pentathionic acid with traces of other acids (34 per cent), and water (19 per cent).

PHYSICAL PROPERTIES OF COLLOIDAL SULPHUR

The sulphur dioxide, formed when the suspension was reduced to a paste, was apparently a decomposition product of the polythionic acids. The amount of the gas was greatly increased when the paste was left exposed to the air for a day. But, the formation of the gas almost ceased when the paste was sealed in an air-tight container. Paste that was exposed to air gradually lost its colloidal properties and was not easily peptized in water. Moreover, the sulphur when completely dried could not be freely suspended in water, even after much tedious grinding. When left in the dry state, the sulphur eventually crystallized into the rhombic sulphur form.

A suspension made up of 1 gm. of paste in 500 cc. of water was creamy white in appearance, free from the odor of sulphur dioxide, and very stable in nature. A suspension kept in the laboratory for 3 months retained its normal appearance and very little sulphur settled out. The colloidal properties were not destroyed by small amounts of lead arsenate and potassium fish-oil soap. It was necessary to dissolve the soap in the water before the sulphur paste was added.

COLLOIDAL SULPHUR PASTE FOR COMMERCIAL USE

The quantities of the materials used and the procedures followed throughout this investigation were chosen without consideration of the time involved. Preliminary tests had shown that, by the methods used, a maximum amount of pentathionic acid could be produced.

In some other tests a slight modification was made by bubbling hydrogen sulphide into a concentrated solution of sulphurous acid. It seemed prac-

ticable to then introduce more of each of the gases, the sulphur dioxide always being in excess, until a thick creamy suspension was formed. A great reduction in the time required for the evaporation was thus effected. It was found, however, that by this short method the relative amount of pentathionic acid was reduced. When, to hasten evaporation, the temperature was raised, the separation of sulphur was increased, but the time required for concentrating the suspension to a paste was greatly reduced. Whether or not the time and labor saved by the use of these methods compensate for the decrease in the relative amount of pentathionic acid in the paste has not been determined.

TOXICITY OF PENTATHIONIC ACID AND COLLOIDAL SULPHUR

Many tests were made to determine the effect of pentathionic acid on 3 biological forms—fungi, bacteria, and insects—all of which may be detrimental to agricultural crops.

Plant Pathogenic Fungi

Nine pathogenic fungi, each of which sporulated readily on potato-dextrose agar, were chosen for toxicity tests. In each case the fungus was transferred to the middle of an agar plate. The plate was then placed in a constant-temperature chamber until the fungus produced an abundant supply of conidia. About 10 cc. of a slightly buffered (pH 5.4) sodium hydroxide-phosphoric acid-mannite solution were poured onto the fungous colony and spread around with a glass rod. The suspension was then diluted with more of the solution until a low-power microscope field contained about 100 spores.

A solution of pentathionic acid, prepared from barium pentathionate, was made twice as strong as was desired for the toxicity tests. Two cubic centimeters of the spore suspension and the same volume of the acid were transferred to a test-tube. By means of a small glass rod a small drop of the spore suspension-acid was spread in a thin film on each of 4 cover slips. The slips were then inverted over glass rings contained in a Petri plate. The excess of the spore suspension-acid was poured into the bottom of the plate. By this means a vapor-pressure equilibrium was maintained within the germination chamber. The set was then placed in a constant-temperature chamber adjusted to 23° C.

With but two exceptions, the percentages of spore germination were determined at the end of 18 hours by counting individual spores under the low power of the microscope. *Verticillium albo-atrum* and *Phomopsis* sp. required 48 hours for satisfactory germination. A spore without, or with a very short dead, germ tube was recorded as not germinated.

The results of the tests show that pentathionic acid is very toxic to the spores of the 9 pathogenic fungi tested, whereas sulphuric acid of the same

TABLE 4.—*Toxicity of pentathionic acid to spores of pathogenic fungi in a buffer-mannite solution*

Pathogenic fungi that were tested	Germination		
	$H_2S_5O_6$		H_2SO_4 .0068 N.
	.0068 N.	.00068 N.	
	Per ct.	*Per ct.*	*Per ct.*
Sclerotinia cinerea	0.0	60.0	97.1
Phomopsis sp.	0.0	67.5	87.4
Verticillium albo-atrum	0.0	20.8	98.6
Graphium ulmi	0.0	8.7	70.9
Glomerella cingulata	0.0	71.3	91.3
Botrytis cinerea	0.0	97.0	98.3
Fusarium monifiliforme	3.3	43.9	89.1
Thielavia basicola	0.0	29.1	69.9
Phoma pomi	0.0	55.1	96.8

normality shows no marked toxicity to the spores of the organisms. The presence of mannite in the solution evidently made conditions more favorable for germination of *Sclerotinia cinerea* spores, and, consequently, about twice the usual amount (6) of pentathionic acid was required to prevent germination.

Plant Pathogenic Bacteria

Three species of bacteria—*Phytomonas pruni, Erwinia amylovora,* and *P. tumefaciens*—were used in the tests. Eighteen to 24-hour-old cultures were used because at this age the organisms were probably at their most active and vigorous stage of growth. The toxicity of pentathionic acid to these bacteria was tested by the Rideal-Walker method (9), modified in a few respects. Instead of only 0.1 cc. of culture 0.5 cc. was introduced into the solution the toxicity of which was to be determined.

The pentathionic acid for the tests was prepared from the barium salt by precipitating out the barium as a sulphate. The toxicity of phenol was determined by the same modified method. The following formula was used in calculating the phenol coefficient of pentathionic acid.

$$\frac{\frac{a_1}{b_1}+\frac{a_2}{b_2}}{2}=\text{Phenol coeff.}$$

In the formula, a_1 and a_2 represent the dilutions of pentathionic acid required to kill in $2\frac{1}{2}$ and 15 minutes, respectively, whereas b_1 and b_2 represent the dilution of phenol in similar tests.

TABLE 5.—*Toxicity of pentathionic acid to Phytomonas pruni, Erwinia amylovora, and P. tumefaciens; and the phenol coefficients*

Name of organism	Dilution of pentathionic acid necessary to kill		Phenol coeff.
	2½ minutes	15 minutes	
P. pruni	1–3,000	1–15,000	77.50
Er. amylovora	1– 500	1– 1,000	6.25
P. tumefaciens	1– 500	1– 2,000	11.46

The grams of the acid and the number of cubic centimeters of water necessary to make the dilutions that were required to kill the bacteria in 2½ and 15 minutes, together with the phenol coefficients, are given in table 5. The results of some preliminary tests indicate that sulphuric acid also is toxic to these bacteria. The toxicity of the sulphuric acid, however, is apparently due to the hydrogen ion, as, in tests in which buffered solutions were used, no toxicity was apparent. On the other hand, pentathionic acid exerted a toxic action 2 to 6 times greater than did sulphuric acid. It is concluded that the pentathionate radical of pentathionic acid is very toxic to these plant pathogens.[2]

Plant Lice

The experiments of O'Kane and Conklin (7) were called to the attention of the writer about the time similar work on fungi had been completed. These entomologists, in their studies on the toxicity of lime sulphur to San Jose and oyster-shell scales, found that there was neither enough hydrogen sulphide nor sulphur dioxide evolved from lime sulphur to kill the insects. The failure of these sulphur compounds suggested the testing of pentathionic acid.

Three common species[3] of aphids—the bean aphis (*Aphis rumicis* Linn.), the cucumber aphis (*A. gossypi* Glover), and the Spiraea aphis (*A. spiraecola* Patch)—were used in tests to indicate the value of pentathionic acid as an insecticide. Small plants or twigs infested with aphids were brought into the laboratory and set up in distilled water. In each test, the host and the aphids on it were thoroughly sprayed with either pentathionic acid, nicotine sulphate, or water. Potash fish-oil soap (0.5 per cent) was used as a spreader in all of the sprays.

About 24 hours after the application of the spray, counts were made of the aphids, those not responding to the touch of a needle point being considered dead. The numbers of insects, dead and alive, that dropped from the plants and of those that remained on the plants are given in table 6.

[2] The toxicity of the acid solutions, prepared by the writer, and of phenol was determined by R. C. Thomas, Department of Botany and Plant Pathology.

[3] Determined by C. R. Cutright, Department of Entomology, Ohio Agricultural Experiment Station, Wooster, Ohio.

TABLE 6.—*Toxicity of pentathionic acid and of nicotine sulphate to 3 species of plant lice*

Species of aphids and the materials tested	Dropped from plant		Remaining on plant		Dead
	Alive	Dead	Alive	Dead	
					per cent
Cucumber aphis on cucumber					
0.02 per cent nicotine sulphate	0	5	9	210	95.9
0.02 " " pentathionic acid	3	7	116	102	45.8
0.05 " " " "	3	12	17	197	91.3
Distilled water check	5	1	197	2	1.5
Bean aphis on Euonymus					
0.02 per cent nicotine sulphate	1	7	9	302	96.9
0.02 " " pentathionic acid	11	7	37	246	84.0
0.05 " " " "	2	14	7	302	97.2
Distilled water check	3	0	180	7	3.8
Spiraea aphis on Spiraea					
0.02 per cent nicotine sulphate	15	15	12	296	92.0
0.02 " " pentathionic acid	9	2	19	347	94.6
Distilled water check	13	10	217	12	8.7

Plants sprayed May 30, 1930, 10 a. m. Counted May 31, 11.30 a. m.

The results of these preliminary experiments indicate that pentathionic acid is very toxic to plant lice. It appears to be about $\frac{1}{3}$ as toxic as nicotine sulphate to the cucumber aphis and equal to the nicotine spray in the control of the Spiraea aphis.

The Effect of Pentathionic Acid on Earthworms

Some preliminary tests on the effect of pentathionic acid on earthworms were made by H. F. Dietz, Research Fellow, Department of Entomology. He found that a 0.5 per cent solution of the acid was capable of killing the worms in an hour. A reducing action of the pentathionic acid was indicated by the bleaching of the worms. He further observed that, from the standpoint of the time necessary to kill, the acid compares favorably with pyrethrum.

Colloidal Sulphur as a Fungicide

On the following pages are given the results of some preliminary tests on the effect of colloidal sulphur on the apple scab (*Venturia inaequalis*), the bitter rot of apple (*Glomerella cingulata*), and the damping-off of tomato (*Pythium debaryanum*), organisms. No conclusions are drawn from the meager results. They are given here merely to indicate some of the possibilities of hydrophilic colloidal sulphur.

Venturia inaequalis

Tests of the toxicity of pentathionic acid to the ascospores of *Venturia inaequalis* were the only ones in which the perfect stage of a fungus was used.

Small apple trees, growing in the greenhouse, were sprayed with colloidal sulphur varying in strength from ½ to 3 lb. to 50 gal. of water. On the following day leaves that had been thoroughly sprayed were brought into the laboratory, washed in a steady stream of water for 10 minutes, and then placed in the bottom of a Petri plate. An old apple leaf bearing mature ascospores was fastened by a wet filter paper in the top of the plate. The leaves were held in this position for 5 hours, during which time ascospores were discharged from the old leaf onto the sprayed leaf. The source of inoculum was then removed and the plate was set away at 23° C. for 24 hours. Apple leaves sprayed with dry lime-sulphur, 3 lb. to 50 gal. of water, and treated in the same way, were employed in control tests. At the end of the germination period the percentage of germination was determined, as described earlier.

TABLE 7.—*The toxicity of colloidal sulphur and lime-sulphur, sprayed on apple leaves, to Venturia inaequalis ascospores*

Tests	Types and concentrations of sprays and percentage germination					
	D.L–S. 3#	C.S. ½#	C.S. 1#	C.S. 1½#	C.S. 2#	C.S. 2½#
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	4.6	47.2	17.3	7.4	2.1	0.0
2	10.6	31.4	21.9	6.4	0.0	0.0
3	5.1	44.0	30.7	2.1	0.0	0.0

The results of 3 quadruple tests, given in table 7, indicate that colloidal sulphur, 1.5 lb. to 50 gal., is as toxic to ascospores of *Venturia inaequalis* as dry lime-sulphur, 3 lb. to 50 gal. It should be remembered that the sprayed leaves were subjected to washing for 10 minutes in a steady flow of water. In the tests neither of the 2 types of sprays caused noticeable injury to the apple leaves.

Glomerella cingulata

Leaves for this experiment were sprayed and treated in the same manner as in the preceding one, except that wettable sulphur instead of lime sulphur was used in the checks. After the leaves were washed, they were sprayed with a spore suspension of *Glomerella cingulata*. After 24 hours, no germination had occurred on the leaves sprayed with colloidal sulphur, 2 and 3 lb. to 50 gal., whereas on leaves sprayed with the same amounts of wettable sulphur over 50 per cent of the spores had germinated. Here, again, no injury to the apple leaves was apparent.

Pythium debaryanum

In an effort to control damping-off of tomato seedlings 3 tests were made. In one test the seeds were dipped in a heavy suspension of colloidal sulphur to which alcohol had been added. The seeds were then dried and planted in soil contaminated with *Pythium debaryanum*. In another test, the soil was thoroughly soaked with a colloidal sulphur suspension. After the soil was dry again, tomato seeds were planted in it.

Although the third test does not include the use of colloidal sulphur, it is included here because Babson (11) has shown that when sodium thiosulphate is applied to soil rich in organic matter about 80 per cent of it is transformed to pentathionic acid. On the basis of this, a 1 per cent mixture of sodium thiosulphate and soil infected with *Pythium debaryanum* was made up and planted with tomato seeds.

In all 3 of the tests the stand of tomato seedlings was improved by the respective treatments. There was some evidence of seed injury in the first and third tests, but it was not severe enough to be a limiting factor.

SUMMARY AND CONCLUSIONS

Sodium, barium, and potassium pentathionates were prepared from concentrated acid formed by the action of hydrochloric acid on sodium thiosulphate. These salts are stable when thoroughly dried and kept at room temperature. In the presence of moisture they break down, forming, among other products, sulphur and sulphur dioxide.

The optical properties of the barium and potassium salts agree with the descriptions and measurements of the pentathionate salts of the elements. The optical properties of the sodium pentathionate have been determined by V. H. Morris.

By gravimetric methods, the various salts were found to be from 95 to 98 per cent pure with reference to the amounts of the basic elements and the sulphur.

On standing, a concentrated solution of pentathionic acid slowly decomposed, forming free sulphur and sulphur dioxide. However, dilute solutions showed only slight decomposition after standing 6 months.

By volumetric titrations ,the acid prepared from barium pentathionate was found to be about 1 per cent trithionic, 3 per cent tetrathionic, and 96 per cent pentathionic.

A suspension of hydrophilic colloidal sulphur, prepared by the sulphur dioxide-hydrogen sulphide method, when reduced to a paste, was found to contain about 47 per cent free sulphur, 34 per cent pentathionic acid, and 19 per cent water.

Sulphur dioxide was formed when the paste was exposed to air. When sealed in an air-tight container, the product seemed quite stable. A spray suspension, 3 lb. of paste to 50 gal. of water, did not smell of sulphur

dioxide. In such a spray the sulphur remained in good suspension for 3 months.

Pentathionic acid was found to be toxic, to different degrees, to 9 pathogenic fungi, 3 bacterial pathogens, 3 species of plant lice, and to earthworms.

Hydrophilic colloidal sulphur was found to be toxic to 3 plant-pathogenic fungi. In consideration of the strength of pentathionic acid necessary to kill or inhibit growth of the organisms and the amount of pentathionic acid in the sulphur paste, the following deductions were made. A spray made up of 3 lb. of paste to 50 gal. of water contained 3 times as much pentathionic acid as was required to prevent germination of the spores of the bitter-rot fungus (*Glomerella cingulata*), 8 times the amount necessary to inhibit growth of the fire-blight organism (*Erwinia amylovora*), and 12.5 times the amount that killed 1 species of the apple aphis (*Aphis spiraea*).

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THE ANATOMY OF THE "KROEPOEK-DISEASED" LEAF OF NICOTIANA TABACUM AND OF ZINNIA ELEGANS

L. C. P. KERLING

(Accepted for publication May 4, 1932)

From Dr. T. H. Thung, phytopathologist at the Experimental Station "voor Vorstenlandsche Tabak" at Klaten, I received leaves of Kroepoek-diseased tobacco plants, used by him in his research on the symptoms and cause of this disease.

The diseased leaves have fantastically bent veins; Jensen (3, p. 51), who gives a short description of this disease, says that the leaf has the appearance "as of being kneaded and wrinkled in a hand, and again partly unfolded from itself." This is the reason that this disease was named "Kroepoek," after a well-known Indian dish. Many investigators have described it. For a complete list of literature I may refer to a paper of Dr. Thung, which will appear shortly.[1]

The cause of the Kroepoek of tobacco is a virus, which can be transferred from diseased to healthy plants by grafting or direct organic connection. The vector was discovered only recently almost simultaneously by Storey (8) at Amani in East Africa and by Thung at Klaten. It is a white fly of the family *Aleurodidae* and the genus Bemisia. The insects could induce the symptoms of Kroepoek on healthy plants in insect-proof houses.

Aleurodidae were also noticed on many weeds, but the identity of these with the ones on tobacco could not yet be settled. Many plants have disease symptoms coinciding with those of the Kroepoek: thus I found at Djokjakarta, in various gardens, zinnias with thickened and curled leaves.

Thung[1] transferred 2 white flies of a diseased zinnia to healthy tobacco plants in a greenhouse. After 14 days the tobacco had the Kroepoek disease. The same virus thus induces the symptoms of the disease on both of these plants.

The appearance of the Kroepoek-diseased leaves has been already repeatedly described by various investigators. A short repetition will here, however, not be superfluous, in order to connect definitely the macroscopic and microscopic symptoms.

MACROSCOPIC SYMPTOMS OF THE DISEASED TOBACCO LEAF

Thung[1] distinguishes 3 principal types, which produce constant symptoms by inoculation, independent of incidental circumstances. Also, in

[1] This paper will be published in *Mededeeling van het Proefstation voor Vorstenlandsche Tabak*. Dr. THUNG was so kind as to give me much information concerning the disease, for which I wish to express here many thanks to him.

transferring them by means of the white flies, each type retained its individuality.

Type a: the most common Kroepoek. The deviations are visible on both sides of the lamina. At the upperside the leaf sometimes exhibits over the whole length a downward-pointed nod due to the curving of the lateral veins of the first order. The midvein also may be curved. The ultimate branches of vascular network of the leaf are locally sunken, so that shallow furrows occur. On the underside of the leaf the curved veins present a fantastic course, their surface is irregular (Fig. 1, B). The more minute veins produce swellings to the end that even the most minute branches become thus involved. The swellings look like round or somewhat elongated, often irregular knobs and the original course of the vein remains visible in the thickening (Fig. 1, A). Sometimes the large and the small

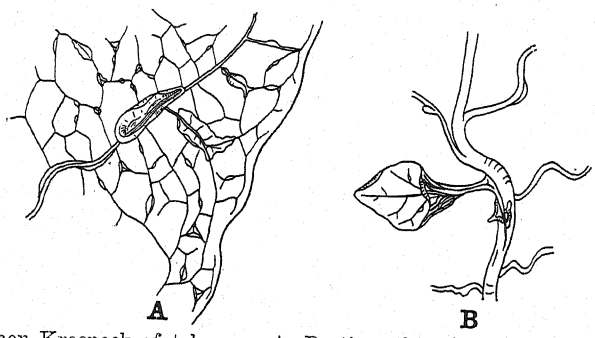


FIG. 1. Common Kroepoek of tobacco. A. Portion of leaf. B. Cup-like outgrowth of the main vein of a tobacco leaf.

veins form together a thickened network. The veins of the first order especially protrude like thick cords above the leaf. The swellings very often enlarge, mostly at both sides of a vein, so that they form a protruding margin, which is loose from the lamina. These margins stand oblique upwards, so that a second lamina, 2–14 mm. long, is formed, adjoining the original one and having with this a common vein (Fig. 1, A). The original lamina and the new one are in all cases situated with their undersides against each other as can be distinctly seen in the veins of the new, secondary lamina. The largest secondary leaflets are chiefly situated near the midrib and at the lateral veins of the first order. They are of very irregular form, sometimes somewhat oblong, mostly elongated along a vein until 18 mm. in length. The margins are irregularly lobed or crisped, often curled and sometimes locally grown together with the vein from which the leaflet issues. In a single case the leaflet transverses the vein. Often a second, smaller lamina develops under the new one, grown together at the

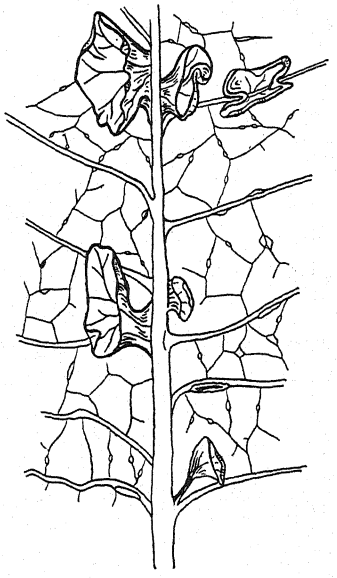


FIG. 2. Leaf- and cup-like outgrowths from the main vein of tobacco.

base and with the margins with the first one, so that a pocket-like appendix arises.

In the strongest deviated cases this distorted part elongates to a flat broad stalk on which a cup-like lamina is situated (Fig. 2). In the extreme cases the stalk is 1 cm. long, narrow, and supports a pocket-like lamina of a leaf form, like that of the original leaf (Fig. 1, B). The old and new laminae are connected only at the junction of the new "petiole." The new laminae have thickened veins similar to those of the diseased original leaf.

Type b: the curl disease. Especially the top leaves have regularly downward-curled margins, whereas the mesophyll between the veins protrudes convexly upwards. The main and lateral veins of the first order are not contorted or bent downward; the thickenings of the vein on the underside are more regularly spread over the whole lamina. This type is not further investigated here.

Type c: the clear Kroepoek. The margins of the leaf are turned upward. The main and lateral veins, which remain shorter than normal, have small thickenings "as if they are somewhat laced by a thread" (Thung). Some tertiary veins look somewhat transparent. Thickenings of the veins and secondary leaflets do not, however, occur here; this type differs from the preceding two.

TECHNIQUE

For the anatomical investigation fresh leaves were cut and also material fixed in alcohol was used. Diseased and healthy leaves of tobacco as well

as of zinnia were always treated in the same way; they were gathered at the same hour, the sections were treated alike. Some sections were treated with I. I. K., others with phloroglucin and HCl, with fuchsin or with gentian violet. Those treated with fuchsin were imbedded in Canada balsam, those with gentian violet in Hoyer's imbedding liquid.

THE "COMMON KROEPOEK," ITS MICROSCOPIC CHARACTERS

a. Portions of a diseased leaf, which show neither external nor anatomical changes. Contradictory to healthy leaves, the diseased ones gathered at 6 o'clock a. m. contain much starch in the spongy as well as in the palisade parenchyma. This phenomenon also occurs in leaves without distinct anatomical deviations.

b. Slight attack of the lamina. The sections were taken of portions attacked as shown in figure 1, A. Whereas the normal lamina has a uniform thickness of $\pm$ 150 μ throughout, the diseased one has at the underside irregular curvatures, and always where vessels are to be found. The thickenings may occur at the ends of those veins comprising only a few vessels and whose diameter is there $\pm$ 200 μ. Under a small vein the normal leaf has 2 to 3 layers of spongy parenchyma cells of irregular form and with large intercellular spaces. The cells of the undermost row are somewhat more connected together and are elongated in the direction of the epidermis, $\pm$ 30 μ long and $\pm$ 15 μ high. The spongy parenchyma cells of the diseased leaf are more connected together, those of the undermost row are elongated at right angles to the epidermis, and are $\pm$ 30 μ high. Thus, a new palisade layer is formed, lower, however, than that of the upper side, which is $\pm$ 60 μ high. Through these changes the lamina curls upward, whereby the afore-mentioned sunken parts on the upper side are produced. The changes of the very small veins are in the main the same as those of the large ones.

c. Slight attack of the midrib and of the lateral veins of the first order. Leaves gathered at 6 a. m. contain a great quantity of starch in the medullary rays, the cambiform cells, in the cells of the pericycle and of the cortex parenchyma. Besides externally healthy looking portions of diseased leaves may already have anatomical deviations, which become more distinct in the neighborhood of the curled parts and of those bearing secondary leaflets.

The pericycle of normal leaves is, at the under side of the stele, not more than 2, at the utmost 3, cells wide. The primary phloem is situated in small groups at regular distances (Fig. 3, A). The diseased leaves have enlargements that arise through strong division of the cambium cells. While a cambium cell of the normal leaf does not form more than 1 to 3

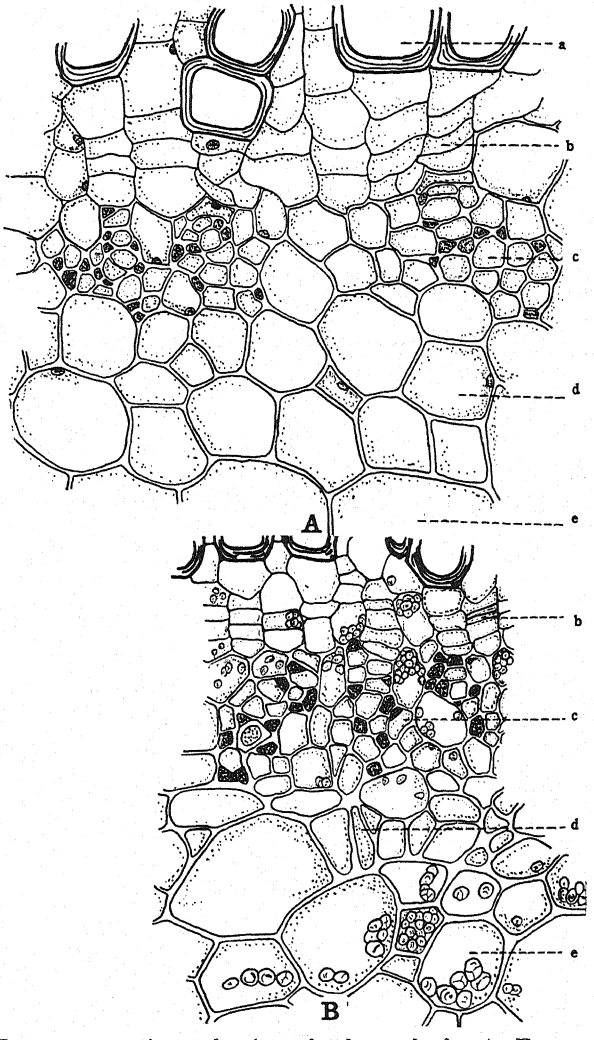


FIG. 3. Transverse sections of veins of tobacco leaf. A. From normal leaf: a, secondary xylem; b, cambium; c, primary phloem; d, pericycle; e, endodermis. ×295. B. From leaf showing beginning of common Kroepoek. ×210. Small letters have the same meaning as in A. However, the phloem was scattered, the pericycle was flattened, and the endodermis contained much starch.

rows of tissue at the outer side, the cambium of the diseased leaf forms radial rows of 5 to 7 cells. The small groups of primary phloem are no longer to be distinguished separately; they are united to complexes of irregularly placed sieve tubes and companion cells (Fig. 3, B). The sieve tubes are sometimes easily recognized by a sieve plate. The cells of the

pericycle also divide irregularly; small cells arise, some of which are strongly flattened. The endodermis of the diseased leaves remains easily distinguishable by the many large starch grains. Those of the healthy full-grown leaf are smaller; also, there are fewer cells with starch grains. At the upper side of the stele the intraxylary phloem behaves likewise; large irregular groups are formed. Because of the strong tension, schizogenous holes may arise in the pericycle (Fig. 4, A). There are here no other changes inside the endodermis.

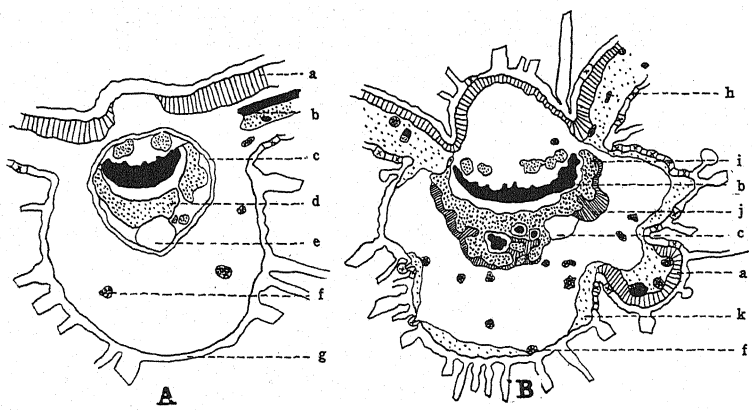


FIG. 4. Transverse sections of veins of tobacco leaf. A. Beginning of attack. ×295. B. Beginning of secondary leaflets. ×215. Small letters have same meaning in both parts of the figure. a, Palisade parenchyma; b, xylem; c, endodermis; d, primary phloem; e, schizogenous cavities; f, crystal cell; g, epidermis with hairs; h, stomata; i, secondary phloem; j, pericycle; k, spongy tissue.

d. Sections of a distinctly deviating part of the main vein. At the under side of the stele phloem proliferations are to be seen at one or more places, which may lead to irregular curving of the endodermis. Between and also within the proliferations, which consist chiefly of small cells with sieve tubes and companion cells, there remain strips of thin-wall tissue adjoining the medullary rays and the ends of the original cambium layer. The cells of these strips are irregularly situated; sometimes small rows of 2 to 3 cells occur.

In the midst of these proliferations small woody vessels arise without forming a distinct cambium. After some time circular cambia occur, producing new secondary xylem against the small woody vessels and outwardly forming secondary phloem. The primary phloem remains outside this. The cambia arise in the above-mentioned tissue strips (Fig. 4, B, and 5).

In a longitudinal section a distinct connection between the cambia of the old and of the new steles was sometimes observed, also the xylem parts may sometimes be connected by a series of small, narrow vessels. In a prolif-

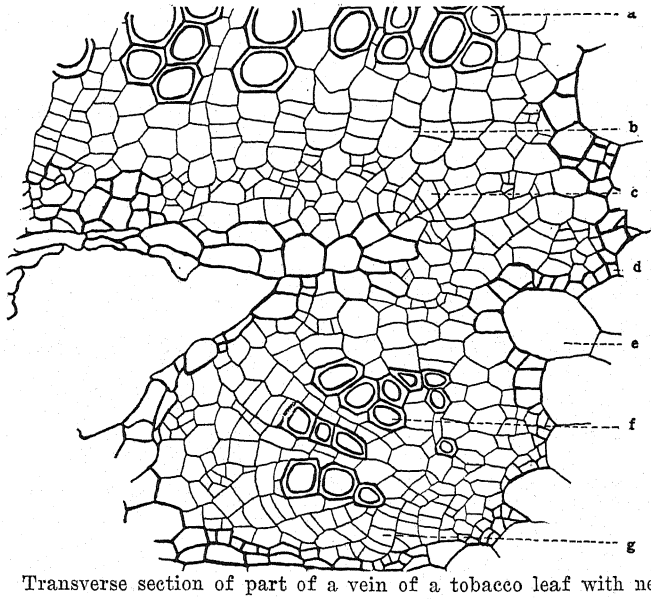


FIG. 5. Transverse section of part of a vein of a tobacco leaf with newly formed stele and schizogenous cavity. ×295. a, Secondary xylem; b, cambium; c, primary phloem; d, rest pericycle; e, endodermis; f, newly formed xylem vessels; g, newly formed cambium.

eration, however, the new strands have, for the greater part, a course independent of the old ones.

The new steles may becomes ± 160 μ in diameter. They remain entirely within the pericycle. As they increase they cause larger tensions in the surrounding tissue; nonproliferating cells of the pericycle are either flattened or torn (Fig. 5). In the latter case large schizogenous holes of

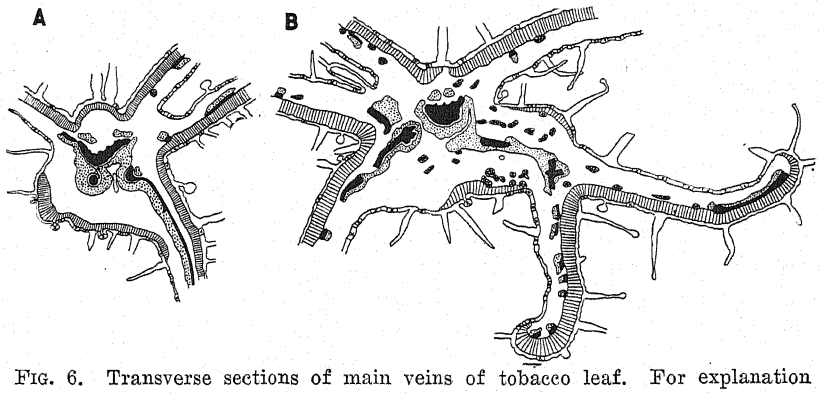


FIG. 6. Transverse sections of main veins of tobacco leaf. For explanation see figure 4. ×25. A. Slightly diseased. At the right there are 2 secondary leaflets. B. Severely diseased. At the left there are 2 loose and at the right 2 joint secondary leaflets.

irregular form arise. The endodermis is now very strongly transformed. If the new steles arise one-sided, then the midrib curves as described above.

The new steles are not always round; often the radial rows disperse flabellately from the point where the first and smallest vessels are found. The cambium of the steles situated at the sides of the vein opens and runs parallel to the original cambium of the main stele (Fig. 6, A). However, the new secondary xylem is formed here at the dorsal side of the leaf and the secondary phloem at the ventral side. In the latter case the secondary phloem adjoins the original primary phloem. In this reverse orientation the new stele proceeds towards the laterally rising secondary leaf (Fig. 6).

The first-formed primary phloem of the proliferations takes the place of the intraxylary phloem of the normal veins. In longitudinal sections the sieve tubes of the original primary phloem appear to have changed beyond recognition: division walls arise (Fig. 7, C). The sieve tubes

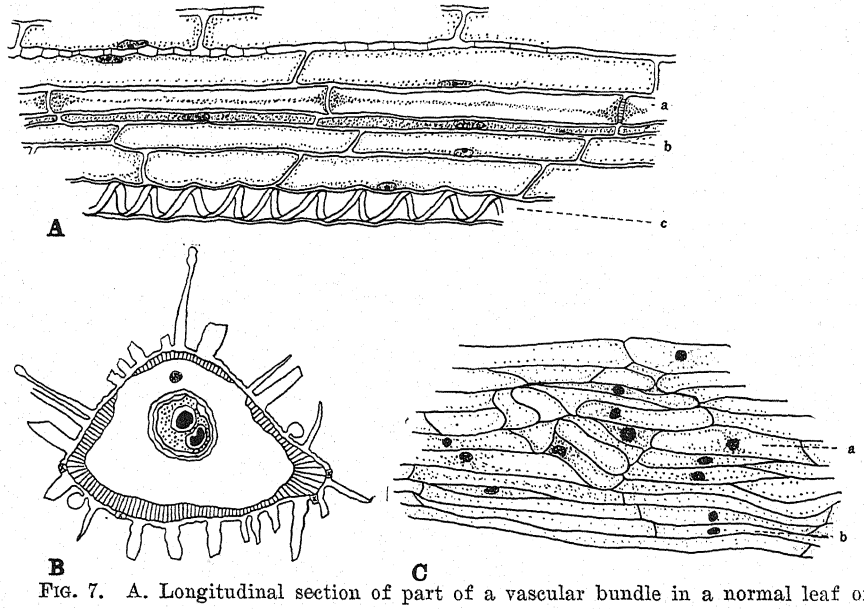


FIG. 7. A. Longitudinal section of part of a vascular bundle in a normal leaf of tobacco. ×295. a, Sieve tube; b, companion cell; c, spiral vessel. B. Transverse section of the stele of the cup-like outgrowth shown in figure 1, B. For an explanation see figure 4, B. ×115. C. Longitudinal section of part of a sieve tube in a new stele. ×450. a, Primary phloem; b, secondary phloem.

formed by the new cambium are elongated and narrow: 3 to 5 μ wide and ± 60 μ long. The sieve tubes of the normal leaf are ± 14 μ wide and ± 140 μ long (Fig. 7, A). The cortex parenchyma of a severely infested part has extended irregularly, especially at the under side where at both

sides sometimes two lobes have developed. They then form the macroscopically visible thickening of the veins between which the original course of the vein remains visible because of the unchanged median part. They are often more developed on one side than the other (Fig. 4, B). It also happens that only one lobe is situated in the median part or at the lateral side of the vein, which may be of very irregular form through the occurrence of folds. The lobes can develop into secondary leaflets that then obtain their above-described irregular arrangement. By the strong extension of the vein on the underside the parts of the original lamina form at both ends of the vein a more acute angle with each other than is the case in the normal leaf. Thus the above-described sunken parts arise. Furthermore, the cortex parenchyma of the upper side is often higher than normal, which probably causes the development of thickened veins on the upper side in the midst of a sunken part.

A normal vein has 5 to 6 rows of collenchyma cells adjoining the epidermis; the middle cells contain a few chloroplasts, those at the sides some more. The epidermal cells are here 30 to 40 μ wide; only a few stomata occur. They are situated in the same plane. The cortex parenchyma cells of the diseased leaf are unchanged, as is the number of crystal cells, but the layer of collenchyma is strongly reduced or has quite disappeared. Especially in the lobes, its place is taken by one or more rows of parenchyma cells containing many chloroplasts.

These cells are closely arranged; those of the outer row are elongated in a direction perpendicular to the epidermis. They form the transition to the palisade parenchyma that develops in the new lamina (Figs. 4, B and 6). Adjoining this tissue small vascular bundles are situated, sometimes at regular distances. They are in connection with the steles inside the pericycle and proceed later in the secondary leaflets. The epidermal cells have divided until they are only 8–12 μ wide. Especially at the lobes, many stomata occur. Sometimes the epidermis exists locally only of hair-like cells and stomata with their adjacent cells. The last ones are directed outwards, so that the stomata themselves protrude above the epidermis (Fig. 4, B). The substomatal cavities are larger than those of the normal leaf.

e. Development of the new lamina. If the two folds develop on both sides 4 new laminae arise. In most cases, however, only one fold on the left and one on the right side develop. Sometimes 2 laminae develop at one side and it may also occur that a lobe develops unequally so that it divides at some distance out on the midrib into 2 lobes. Thus the phenomenon of the stalked new lamina occurs (Fig. 1, B). If the splitting of the lobe takes

place only in the middle and the margins keep together, then the new lamina assumes the pocket or cup-like form, as described before.

The structure of the secondary leaflets is like that of the original lamina. They are, however, always situated with the similar tissues towards each other and towards the old lamina. The leaflets just under the original leaf turn the spongy parenchyma upwards, the palisade tissue downwards. The above described new steles, which are also situated upside-down, proceed in the leaflets and form the veins (Fig. 6, A). If a second leaflet lies under the first secondary leaflet, then it has again the position of the old leaf, and the veins are situated normally. In all cases the xylem part is turned towards the morphological upper side of the leaflet, the sieve part towards the under side.

MICROSCOPIC CHARACTER OF THE CLEAR KROEPOEK OF THE TOBACCO LEAF

Leaves gathered early in the morning are quite free from starch. In transverse sections the primary venation shows irregular swelling on the under as well as on the upper side. In these swellings the xylem is wider because of the marked division of the cambium that protrudes there. In longitudinal section this protrusion appears to be caused by the swellings of the ends of the cells of the xylem vessels (Fig. 8, A). The swollen ends of two cells are situated against each other. Also the secondary phloem is strongly developed. In contrast to the normal leaf (Fig. 3, A) a great number of sieve tubes and companion cells are here already formed (Fig. 8). The dimensions of these sieve tubes agree with those of normal ones. The primary phloem is flattened strongly, the sieve tubes are difficult to relocate in transverse sections. In longitudinal sections the cells are curved and often very narrow, except at the sieve plates. The walls are irregularly thickened (Fig. 9). The pericycle of the normal leaf is $\pm$ 60 μ wide; that of the diseased leaf $\pm$ 250 μ, which is due to the enlargements of the cells up to 120 μ (Fig. 8, B). Between these strongly enlarged cells also flattened ones occur.

The gigantic cells possess thin walls; sometimes the wall is still thickened at one side. The smaller cells have thick walls; the intercellular spaces are filled with the same yellow substance as that of the cell walls. The endodermis is hard to find, while starch grains occur only sporadically.

The cortex parenchyma also shows local elongation of the cells in radial direction. Through this lengthening process other cells are sometimes flattened. In the enlarged cells division walls sometimes occur. The enlarged cells are often the most greatly swollen laterally. The cells turn these sides towards each other, so that local elevations are formed, causing the epidermis to protrude (Fig. 8, A). The result of this is the formation

of the irregular surface of the veins. The large cortical cells also are present in the veins of the second order and in smaller ones through which they obtain the "clear" appearance. The collenchyma in the midrib remains unchanged; it accompanies the epidermis, which covers the swellings. In the smaller veins the collenchyma has disappeared; also the epidermal cells may be somewhat enlarged.

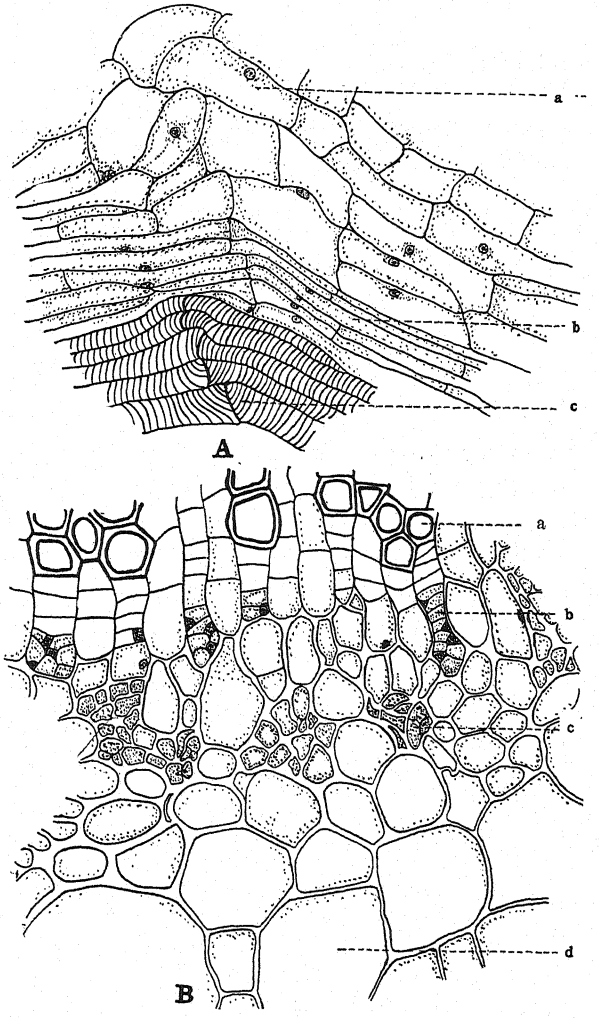


FIG. 8. Sections of veins of tobacco leaves with transparent Kroepoek. A. Longitudinal. ×115. a, Cell proliferation in the pericycle; b, secondary phloem; c, swollen cells of xylem vessels. B. Transverse. ×210. a, Secondary xylem; b, secondary phloem; c, flattened primary phloem; d, enlarged pericycle cells.

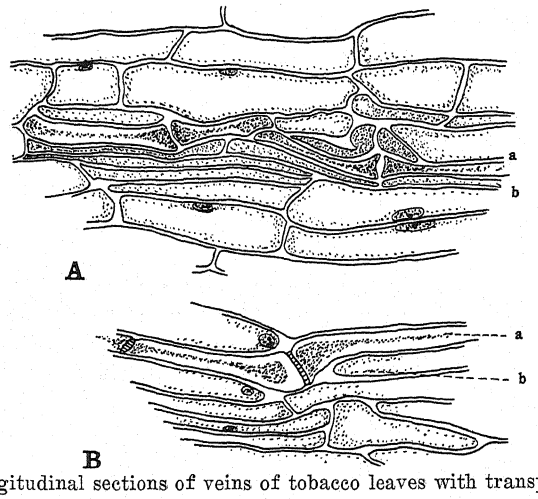


FIG. 9. Longitudinal sections of veins of tobacco leaves with transparent Kroepoek. ×295. A shows transformed sieve tubes of the primary phloem. B shows a swollen sieve tube. In both parts of figure: a, sieve tube; b, companion cell.

MACROSCOPIC CHARACTERS OF KROEPOEK-DISEASED LEAVES OF ZINNIA

The stems and leaves remain abnormally short; besides, the leaves are broader, thicker, and rougher. The cordate lobes are often so strongly developed that the lobes of a pair of opposite leaves are pressed against each other in folds and against the stem.

On the upper side the diseased leaves are lighter green than the healthy ones; sometimes only interveinal lighter spots occur. These parts protrude, then become convex upwards, while the dark green veins are sunken. This phenomenon is very striking, especially at the leaf base. The whole leaf becomes wrinkled.

On the underside the color of the lamina is a pale greyish green, sometimes silvery and shining. The finest veinlets and sometimes also some larger veins are less conspicuous than in the normal leaf, while they are sunken in the thickened mesophyll; they are situated in a furrow. In most cases, however, the larger veins are swollen, especially those of the first and second order. They lie then like thick cords on the lamina. Where the swellings develop locally or are laterally situated the veins take on a crooked course.

The disease symptoms can be found throughout the whole plant, sometimes also in the bracts of the involucre and even in the petals.

MICROSCOPIC CHARACTERS OF THE DISEASED ZINNIA LEAF

Whereas normal leaves (Fig. 10, A) are uniformly thick ($\pm 250\ \mu$), the diseased ones are irregularly swollen, especially in the veins, to a thick-

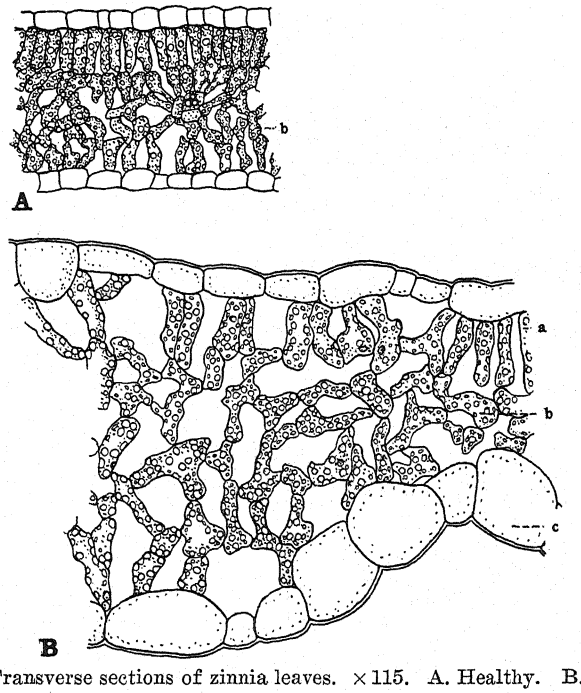


FIG. 10. Transverse sections of zinnia leaves. ×115. A. Healthy. B. Affected with Kroepoek. a, Palisade tissue; b, spongy tissue; c, epidermis. Note that in B the palisade tissue is spongy and epidermal cells are enlarged.

ness of ± 500 μ (Fig. 10, B). The epidermal cells of the normal leaf measure ± 30 μ, those of the diseased leaf are enlarged to ± 130 μ. The healthy leaves possess at the upper side 2 rows of palisade parenchyma cells with small slit-like intercellular spaces; under these occurs a loose spongy tissue of 3 to 4 rows of cells. The diseased leaf does not show much difference in structure between the under and the upper sides. The palisade parenchyma has more rounded cells than has the normal one; the cells are not perpendicular to the epidermis, but are situated obliquely and are separated one from another. Especially the cells of the second row have fantastic forms and are not to be distinguished from those of the underlying spongy parenchyma. The large intercellular spaces and epidermal cells give the leaf a light green color. Also, the spongy parenchyma cells are more dispersed and are larger than normal. Beneath a small vascular bundle the cells of the mesophyll are flabellately arranged; those of the diseased leaf separate and cause the semicircular swellings, as seen in section. The sections of the large veins are nearly square on the under side. The stele shows the same changes as those described for the common

Kroepoek of the tobacco plant; besides the original xylem and phloem numerous new steles, sometimes as many as 9, are to be found inside the pericycle. Also, here the primary phloem has increased strongly and the pericycle is enlarged through cell division. The cells are very small. Each stele has a ring-like cambium. In the vein, however, neither palisade parenchyma nor secondary leaflets develop.

In fresh sections sometimes inclusions of a brown granular substance occur. The particles are globular and are situated sometimes in, sometimes between the cells of the diseased vein.

SUMMARY

Both types of Kroepoek of the tobacco differ from each other not only externally but also anatomically, although they are transferred by the same Aleurodide. The permanence of two separate types in the infection experiments points to the fact that two different species of virus are concerned. The "transparent Kroepoek" produces in the veins typical swellings of the ends of the xylem vessel- and sieve-tube cells and of the cells of the pericycle and of the cortex parenchyma. The sieve tubes are curved: the cell walls of the primary phloem as well as those of the pericycle are irregularly swollen. Much secondary phloem is present, which still seems to function.

The "common Kroepoek" is so very different that it remains a question whether the type with the transparent veins may even be considered as Kroepoek. The common Kroepoek of the tobacco and the Kroepoek of the zinnia agree in many respects. That of the zinnia can therefore be transferred to tobacco by Aleurodidae. In the following points the anatomical deviations agree.

1. Disturbed transport of sugar in the whole leaf, even in the externally healthy looking parts.
2. Increase of the primary phloem in the veins.
3. Enlargement of the pericycle through cell division.
4. Formation of new woody vessels, surrounded by a cambium so that new steles arise inside the old pericycle.
5. Loss of the dorsoventrality of the leaf: In tobacco through formation of palisade parenchyma instead of spongy parenchyma; in zinnia through formation of a loose, irregular palisade parenchyma with large intercellular spaces.

Besides, palisade parenchyma and stomata are formed in the lobed veins of the tobacco; later, the lobes develop into secondary leaflets in which the new steles proceed in reverse orientation. The new leaflets lie with the morphological underside against the underside of the old leaf.

If more lobes are developed, then the similar sides of the leaflets are always directed towards each other.

Remarkable is this formation of a new palisade tissue and of new laminae. The increased assimilation can only augment the quantity of starch. The quantity of calcium oxalate is not changed; sections of diseased and of healthy veins contain an equal number of cells with crystals. This is in agreement with the experiments performed at the Experiment Station of Vorstenlandsche Tabak, which proved that the proportion of inorganic substance of the Kroepoek-diseased leaf remains unchanged (Thung).

The phenomenon of doubling of the lamina has long since been described. Morren (5) enumerates a number of cases in which secondary leaflets develop from the midrib, being directed with the corresponding sides towards the normal leaf. Schimper (7, p. 74) described a tobacco leaf with a stalked cup upon the midrib. Penzig (6) also mentions doubling of the lamina of tobacco with leaf reversion (Bd. III, p. 82); and he notes plants of other kinds with new formations on the midrib of the leaves both ventrally and dorsally, "welche entweder die Gestalt von flachen oder concaven, bandförmigen Blättchen, oder die von röhrigen, trichterförmigen, oben offenen, oft langgestielten Ascidiën haben." (Bd. II, p. 106). This description quite agrees with the common Kroepoek of the tobacco. The cause of these teratologies, named by Masters (4) under "Multiplication" and by Janse (2) under "splittingphenomena" is variously sought. Janse supposes a deviation of the growth enzymes. A too large quantity might cause local proliferation. However, he does not solve the problem. Also Penzig looks for the cause in growth substances, which might give rise to abnormal formations under influence of external stimuli such as parasites or feeding abnormalities. Also Jost (1), on account of experiments of Goebel supposes that in the first place feeding affects the symmetry of the leaf.

The question as to what is the cause of the teratologies is now in this case brought nearer to solution. The virus, transferred by the named Aleurodide causes the splitting of the tobacco leaf and also the loss of dorsoventrality of the zinnia leaf. However, this does not in the least explain the function of the virus in the plant. The first visible deviation is an accumulation of starch. Perhaps in connection with this is an abnormal behavior of the sieve tubes. Also it is possible that the virus influences the growth substances or the enzymes present in the tissues. Which part the accumulation of food plays on the formation of the new steles and on the laminae and thus on the local loss of dorsoventrality has still yet to be investigated.

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CYTOLOGICAL INVESTIGATIONS ON THE SPIKE DISEASE OF SANDAL, SANTALUM ALBUM

M. J. NARASIMHAN[1]

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INTRODUCTION

It is 35 years since the investigation of this disease, which has been estimated to cause a loss of nearly 6 to 7 lakhs of rupees in Southern India, engaged the serious attention of scientists and forest officers. Many theories had been put forward to explain the etiology of the spike disease of sandal, *Santalum album* L., by various workers, based on field observations, but investigations accurately carried out were first taken up by Butler (4) and Barber (2). A detailed description of the symptoms of the disease, both macroscopic and microscopic, was given by Coleman (5). Basing his conclusion on the evidence obtained from a large number of graft-infection experiments, he was the first to put forward the view that the spike disease of sandal may be of the nature of a virous disease. As opposed to this view, a few workers sought to elucidate the problem by the hypothetical consideration that the disease was the outcome of environmental factors. Hole (11) formulated an hypothesis that an "unbalanced circulation of sap," resulting from a variety of external factors, such as forest fire, unsuitable host plants, drought, etc., led to the outbreak of the disease. No doubt the importance of unfavorable external conditions as predisposing factors in relation to the appearance of the disease is universally recognized, but the significance of the term "unbalanced circulation of sap" is too vague for any plant physiologist to define. Howard and Howard (13), working with diseased peach trees, thought that improper grafting itself was the cause of the development of small leaves of spike and opposed the view held by Coleman (5) that spike disease could be transmitted by grafting. But this method of transmission has been tried by workers all over the world, as in the case of mosaic disease of hops, potato leaf roll, peach yellows, and other virous diseases, so much so that the objection cannot be taken as a serious one. In establishing the transmission of mosaic disease in hops, Thrupp (28) says "there is no evidence that the process of grafting can of itself in any way induce mosaic symptoms to appear in healthy susceptible hops."

[1] The writer wishes to acknowledge his indebtedness to Dr. Leslie C. Coleman, Director of Agriculture, in Mysore, for the encouragement and helpful suggestions given in the course of the investigation.

SPIKE DISEASE OF SANDAL, A VIROUS DISEASE

One of the recognized characteristics of virous diseases, in general, is that they are infectious and are transmissible by mechanical means. While some of the diseases, like the tobacco mosaic, possess a high degree of infectivity and are readily transmissible even at dilutions of 1–10,000, as shown by Allard (1), and are known to be filter passers, others among the group have not been found to be so highly infectious. In the case of sugar-cane mosaic, Brandes (3) found that the disease could be transmitted by rubbing the extract from diseased plants into punctures made in healthy plants, but the percentage of infection obtained was small. Sein (24), however, claims that 95 per cent of the plants were successfully infected when healthy sugar cane was punctured with fine entomological pins through a superposed layer of mosaic leaves. On the other hand, potato leaf roll, aster yellows, and infectious chlorosis of Abutilon, recognized as virous diseases, can be transmitted by grafting, but all attempts at transmission with the extract from diseased tissue have failed hitherto. Transmission of virous diseases by insects has been found in a large number of cases.

In the case of the spike disease of sandal successful transmission by means of grafting was observed by Coleman (5). It was noted that the disease was transmitted only in cases where the stock and the scion were organically united. Other methods, such as budding, leaf insertion (27), have also been reported to be successful in transmitting the disease. Inoculation of the disease extract into healthy plants has always failed to produce the disease symptoms. Punctures made with fine needles, the method adopted by Sein (24) for the transmission of sugar-cane mosaic, proved unsuccessful, but in one instance the plant began to develop mosaic symptoms. With our present knowledge, it may be assumed that the infective character of the spike disease of sandal is comparable to that of potato leaf roll and of peach yellows, which are transmitted only by grafting, warranting us in assuming that it is a virous disease. Cytological evidence to support this view, first put forward by Coleman (5), has been obtained by the writer's finding of intracellular inclusions in the tissues. Investigations on the nature of these intracellular inclusions are embodied in the present paper.[2]

HISTORICAL

Intracellular bodies have been known to occur in many of the virous diseases of animals such as vaccinia, rabies, lymphocystic disease of fish, contagious epithelioma, and sheep pox. Out of nearly 190 different plants, recognized by plant pathologists to be affected by virous disease, in only a

[2] A preliminary note on the subject by the writer was published in PHYTOPATHOLOGY (19).

few of them have the characteristic intracellular bodies been found. Simultaneously with the finding by animal pathologists of the Negri bodies in the hippocampus of dogs affected with rabies, Iwanowski (14) found in the tissues of tobacco mosaic amoebiform bodies closely attached to the nuclei, which he described as "parasitischen Amoeben erinnerende Plasmaanhäufungen." Goldstein (7) later made a careful cytological study of these bodies. Similar bodies, which the author described as X bodies, were found in the cells of Dahlia affected with mosaic and dwarf (8). Kunkel (15, 16) found intracellular bodies associated with the mosaic disease of corn, Fiji disease of sugar cane, Hippeastrum mosaic, and mosaic on Chinese cabbage and *Brassica pekinensis* (Hour). McKinney, Eckerson, and Webb (18) described inclusion bodies in the cells of rosette disease of wheat. Similar bodies were found by Smith (25) in the cells of Petunia, *Phytolacca decandra* L., *Euonymus japonica* L., and *Datura stramonium* L., affected by mosaic disease. Hoggan (10) found X bodies and striate material in a number of solanaceous plants affected with mosaic. In the pericyclic region of the young roots of strawberry dwarf plants, Plakidas (20) found that cells showing degeneration contained one to many amorphous intracellular bodies, staining black with iron alum haematoxylin. Similar structures were reported by Rawlins (21) in the pericycle of the root tips of sugar beets affected by curly top. Thus, it will be seen that inclusion bodies have been found in only 15 of the virus-affected plants.

MATERIALS AND METHODS

Material for the investigation was obtained from naturally infected areas in Uttarhalli, Bangalore, and Chamundi Hill, Mysore. The peculiar pendulous branches of spiked sandal, called the "willow type of spike," occurring at Hirehally, Tumkur, were also collected for examination. A comparative study was made of diseased material obtained by graft infection of healthy sandal plants. Leaves and petioles were fixed in various fixing solutions, such as weak Flemming's solution, Zenker's solution, Schaudinn's sublimate alcohol, and Carnoy's fluid. Sections, 5 to $7\frac{1}{2}$ μ thick, were cut, and stained in Haidenhain's iron-alum haematoxylin and counterstained with anilin safranin or orange G in clove oil. Several other stains also were tried. Successful differentiation was obtained with Flemming's triple stain, picric methyl violet, and Goodpasture's carbol-anilin-fuchsin method. Likhité's (17) method of fixing in 2 per cent osmic acid and examining the sections without staining was not found suitable, as the precipitates formed interfered with the examination. Fixation with Flemming's weak solution was found to give the best results.

The cell contents were studied both in free-hand and microtome sections. Free-hand sections of fresh material were placed in picronigrosin for 24 hours and mounted in glycerine. The cell nucleus stained grey and the bodies yellow. Many vital stains were tried, but success was achieved only with a rapid staining in 0.5 per cent neutral red, the nucleus staining red and the body reddish yellow.

Microtome sections of spiked sandal leaves generally show the presence of intracellular bodies in almost every cell (Fig. 1, A and C). They are very prominent in sections of mature spiked leaves, in which much of the green chlorophyll has deteriorated. The bodies have not been found near the growing point and in the first pair of leaves next to it but can be distinctly made out in the 3rd pair of leaves. In an affected cell generally only a single body is present in close juxtaposition with the nucleus, but very often 2 bodies are seen adpressed to the nucleus 1 on either side. As many as 6 bodies have been observed surrounding the nucleus in some of the cells. Rarely the body is found free from the nucleus. In cases where more than 1 body is found in a cell, 1 to 2 of the large ones may be found adpressed to the nucleus, but the small bodies are to be made out scattered in the cell free from the nucleus.

In sections of spiked leaves, it is usual to observe in the palisade cells, that the nucleus and the body do not occupy a central place, but more often the nucleus is found wedged in between the body and the cell wall bounding the long axis of the cell (Fig. 1, A). Occasionally the body is found completely enveloping the nucleus.

As has been noted by various workers, in investigating other virous diseases of plants, the intracellular bodies in sandal spike are best made out when stained with Haidenhain's iron-alum haematoxylin. In leaf sections stained with this stain (longer method), the bodies stand the destaining with iron alum longer than the nucleus. Kunkel (15) in the case of Fiji disease of sugar cane, and McKinney *et al.* (18), in the case of rosette of wheat, found that the bodies were more easily destained with iron alum than were the nuclei, but Rawlins and Johnson (22) reported the reverse to be the case with the bodies found in tobacco mosaic. In sections of spiked leaf stained with Haidenhain's iron alum haematoxylin (shorter method), the destaining with iron-alum was more rapid in the bodies than in the nucleus. After 24 hours of staining the bodies were found to withstand the action of iron alum for nearly an hour. Counterstaining with orange G in clove oil brings out the structures in good relief. Counterstained with anilin safranin, the structural details in the bodies are better made out.

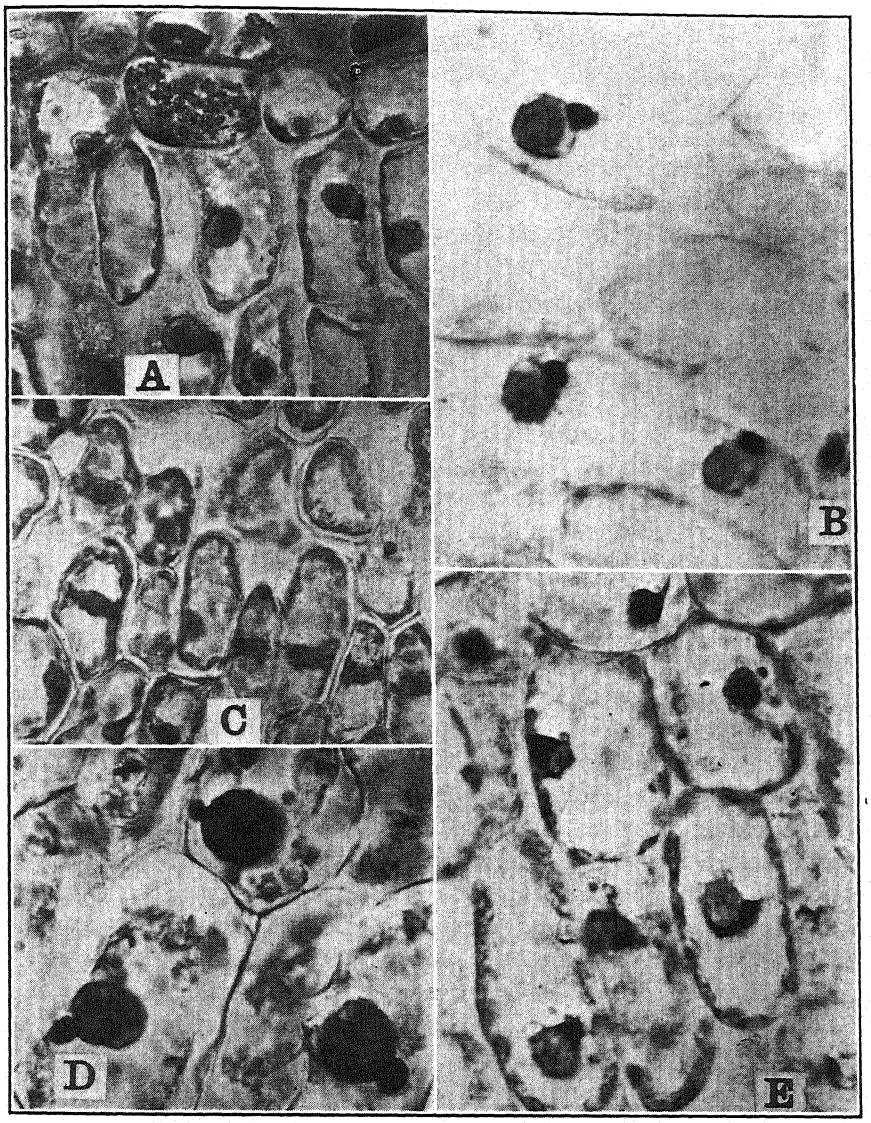


FIG. 1. Photomicrographs of sections of sandal-spike leaves. A. Deeply stained intracellular bodies attached to nuclei. ×820. B. Intracellular bodies with large central vacuoles and other small ones. The membranes around the bodies are evident. The black attached bodies are nuclei. ×980. C. Band-like intracellular bodies across the cells. ×740. D. Large-size intracellular bodies showing vacuolation ×980. E. Proliferation of bodies. ×820. A was stained by Goodpasture's method and B to E with iron-alum haematoxylin.

With Flemming's triple stain, the staining reaction of the bodies has not been found to be specific, as it seems to vary with the duration of staining with gentian violet and orange G. If, after staining with safranin, the sections are stained for a short period with gentian violet, rapidly carried through the higher grades of alcohol, and finally counterstained with orange G in clove oil, the nuclei are stained red; the bodies, orange yellow. If, however, the sections are stained longer in gentian violet, destained in alcohol, and subsequently stained with orange G in clove oil, the bodies retain the violet color. The different procedures adopted in the technique of triple staining by investigators, in general, may perhaps explain the difference reported by various workers in reactions of the intracellular bodies to this stain. Kunkel (16) reported that the bodies in Hippeastrum mosaic showed a special affinity for the orange G stain. The same reaction was observed by McKinney *et al.* (18) in the case of the bodies associated with the rosette of wheat. Rawlins and Johnson (22), however, found that, with the triple stain, the bodies associated with tobacco mosaic became pink. The bodies found in the case of sandal spike seem to react both to gentian violet and orange G, no special affinity being found for the latter stain.

The bodies are made out also by staining with dilute methyl violet followed by treatment with picric acid. This method was found to be successful by Schaffnit and Weber (23) in the case of the intracellular bodies associated with beet mosaic. Sections are transferred from distilled water to dilute methyl violet solution (59 parts of distilled water to 1 part of 1 per cent methyl violet), stained for 2 to 12 hours, rinsed in distilled water, allowed to stand for a minute in picric acid solution (80 parts saturated aqueous solution, 20 parts of 96 per cent alcohol), and dried between filter papers, cleared in xylol, and mounted in balsam. The body takes a grey color, while the nucleus takes a violet color. If the sections, after treatment with picric acid solution and removal of all excess water, are rapidly dehydrated with absolute alcohol, cleared in xylol, and mounted in balsam, the nucleus takes a deep blue color, the bodies a light violet one.

Sections of the spiked leaf also have been stained according to the technique followed by Goodpasture (9) for detecting the cell inclusions associated with rabies, and epithelioma contagiosum. They are stained, first, with carbolanilin fuchsin (0.5 gm. basic fuchsin; 100 cc. 20 per cent. alcohol, anilin oil 1 cc., phenol 1 cc.) for 10 minutes, decolorized with 95 per cent. alcohol, till the sections become pink, washed in water and counterstained with Löffler's methylene blue for 30 seconds, dehydrated in absolute alcohol, cleared in xylol, and mounted in balsam. Destained in 95 per cent. alcohol, the red color persists only in the bodies, even after all the other structures of the cell have lost color. The nuclei are stained blue, and the bodies, a cherry red to dull red. The bodies appear to have an affinity for fuchsin.

None of the previous workers on virous diseases of plants seems to have tried this staining method.

The bodies, associated with sandal spike, are generally round or oval. Often the outline of the body is wavy, with small pseudopod-like projections. In some cells the bodies are found extending from the nucleus, which is adpressed to the cell wall, to the opposite wall, thus appearing to form a band across the cell (Fig. 1, C). Cases have been observed in which the bodies have the appearance of a plasmodium, enclosing the nuclei in their folds. The presence of a membrane surrounding the body has definitely been noted. This is quite evident in cases where the contents of the body has shrunk (Fig. 2, B, body at extreme left). In some of the cells, owing to the disappearance of the contents of the body, the outer membrane persists as a ring attached to the nucleus. In certain instances, where the body presents the appearance of a mere mass of alveolar protoplasm enveloping the nucleus, the presence of a membrane is difficult to make out. Kunkel (16) found no evidence of a membrane surrounding the bodies associated with Hippeastrum mosaic, whereas the presence of a membrane has been reported in the case of the bodies of wheat rosette (McKinney, *et al.*, 18) and tobacco mosaic (Goldstein, 7). The bodies are generally composed of a recticulate matrix, containing one or many vacuoles of various sizes. There is either a large central vacuole surrounded by a thin layer of the body substance or, as is sometimes the case, there are one to two large vacuoles and numerous small ones (Fig. 1, B). As many as 11 vacuoles have been noted in some of the bodies (Fig. 1, D). Kunkel (15) observed in the bodies associated with the Fiji disease of sugar cane a process of division in a plane parallel to the short axis of the cell. Goldstein (7) found that the bodies in tobacco mosaic divide by constriction and the ones in Dahlia mosaic divided and are distributed by the divisions of the host cells. Likhité (17) also reported the occurrence of fission in the case of the bodies of tobacco mosaic. In sections of the petiole of the spiked leaf small bodies are occasionally seen that show a constriction in the middle, but it is impossible to say whether this can be considered a process of division. In addition, it is often to be observed in sections of some of the spiked leaves that the bodies show a large number of proliferations (Fig. 1, E). Figure 2, A, shows a body with 3 proliferations in focus. As many as 7 proliferations have been observed in some of the bodies. One is tempted to believe that the existence of tiny bodies distributed in some of the cells is due to this proliferation. These tiny bodies are stained black with iron-alum haematoxylin and bright red with carbol anilin fuchsin.

Measurements of the bodies along their greatest diameter show a variation ranging from 4.3 μ to 8.7 μ. The bodies were patiently watched under the microscope to see if there was any sort of movement as described by

other workers of mosaic diseases. Goldstein (7) found in the living cells of mosaic tobacco that the bodies changed their form and position frequently. In sections of spiked sandal, kept for a long time under observation, the orientations of the large vacuole or vacuoles in the bodies may be noted. Often the tiny vacuoles fuse together. Very rarely, in some instance a small projection is put forth slowly, which after a time is slowly withdrawn into the body, the entire process taking about 2 hours. It is, however, difficult to estimate correctly the significance of these movements. As Cowdry (6; p. 146) says, "the question always arises as to whether any movement seen, is their own, or that conferred upon them by mass movements of the cytoplasm." Mechanical strains, such as those induced by placing the cover slip over the section mounted in water, are likely to set up conditions other than those obtained in the living cell, which may perhaps result in evoking such phenomena.

THE DISEASED CELL

The effect of the disease on the contents of the leaf is very marked. Butler's (4) observation that the palisade cells elongate at right angles to the surface much more in the case of the spiked leaf than in that of the healthy one was not confirmed by Coleman (5). As the latter investigator pointed out, there seems to be no striking difference with regard to the size of the cells of the leaves of the same age, whether they be healthy or diseased. The writer's observations confirm this view, as will be made out from Figures 2, D and E. In young healthy leaves, the chloroplasts are found in abundance (Fig. 2, E). In old leaves also, these are to be made out lining the cell wall all around (Fig. 2, D). In spiked leaves, the chloroplasts seem to undergo a process of disintegration. During the early stages of the disease, the chloroplasts have a vacuolated appearance, showing clear spaces in their matrix. In very old spiked leaves, they appear as disorganized, shrivelled structures, adpressed to the cell wall. It is in such cells that the bodies stand out prominently. The presence of minute grains of starch in the leaves has been noted by Coleman (5). While, in the fibrovascular sheaths in the leaves, large starch grains are to be found, those found in the chloroplasts are minute. To make out the disposition of these grains within the chloroplasts, it is preferable to select the early stages of spike, as in the older leaves the chloroplasts have disintegrated. The starch grains are clearly brought out by staining with Němec's gentian violet method[3] and are stained bright blue, cytoplasm being grey. Eight to 11

[3] Sections of material fixed in Flemming's weak solution are placed from water in 2 per cent tannin for 30 minutes, washed for 1 minute in water, then transferred to 1½ per cent tartar emetic solution for 10 minutes, washed in water for 5 minutes, stained in 1 per cent gentian violet for ½ hour, rapidly dehydrated, cleared in xylol, and mounted in balsam (see Tunmann (29), 505–506. 29 pp.).

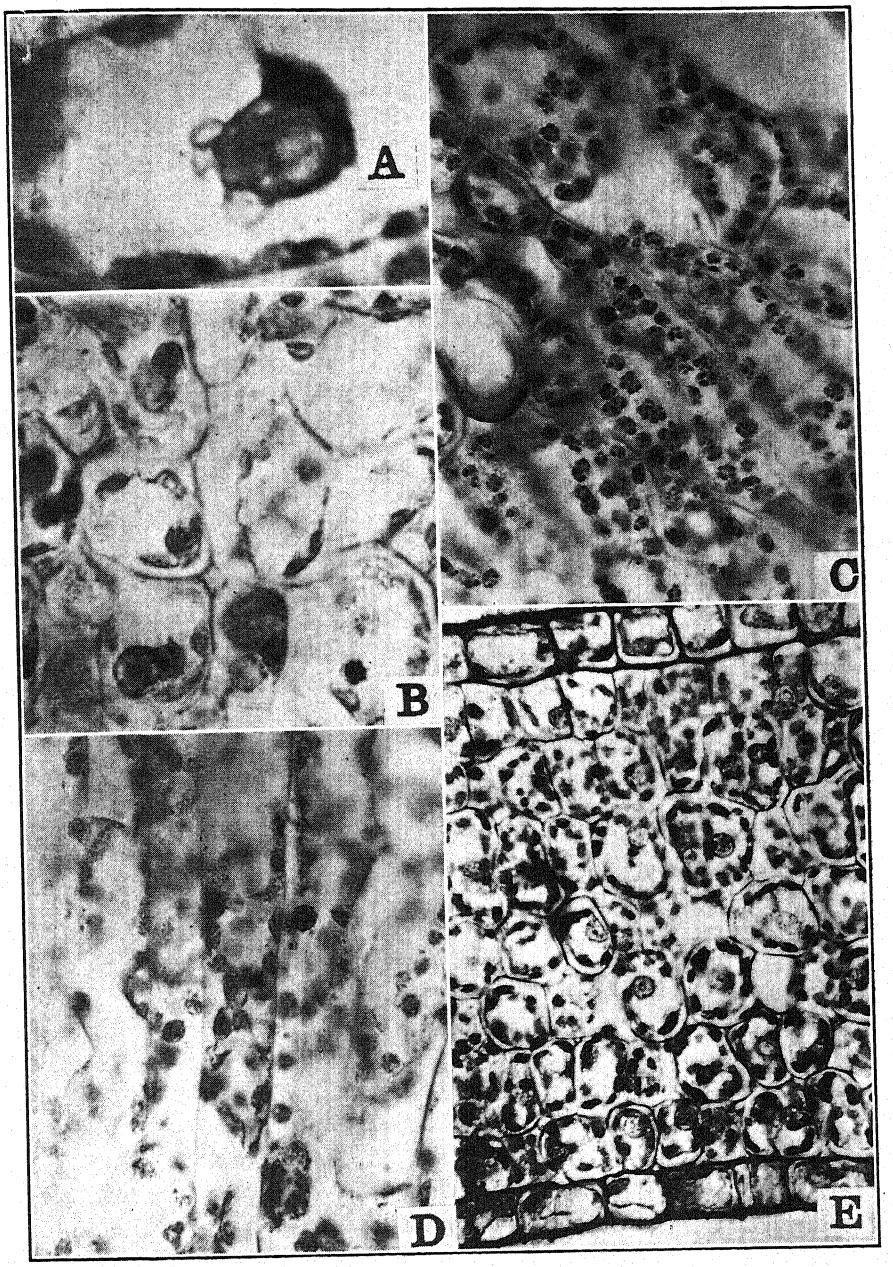


FIG. 2. A–C. Photomicrographs of sections of sandal-spike leaves. A. Highly magnified body with a reticulate structure from section shown in figure 1, E. There are three proliferations at the left. ×1,620. B. Intracellular bodies in the central region of the petiole. ×810. C. Minute starch grains in the chloroplasts. Stained with Němec's gentian violet. ×1,080. D and E. Photomicrographs of sections of healthy sandal leaves. D. Mature leaf, showing elongated palisade cells with nuclei and chloroplasts. ×1,080. E. Young leaf, showing nuclei and abundant chloroplasts stained with iron-alum haematoxylin. ×810.

grains have been found embedded in each chloroplast. Both round and rod shape grains have been noted in the same chloroplast (Fig. 2, C). In fact, they present the appearance of autochthonous starch formed generally in the chloroplasts of leaves; only, it is in a more intensified form. It is possible, as Butler (4) supposed, that the chloroplasts are stimulated by the virus to manufacture more starch than is necessary, the excess being stored in the parenchymatous tissues of the shoots.

The correctness of this view can be tested only after conducting experiments on the physiology of healthy and spiked sandal leaves. The nucleus in affected cells more often shows a dented appearance on the side where the body is attached. In a spiked leaf section some of the few cells in which bodies are not present have normal round nuclei, as in healthy leaf cells. On the other hand, some of the nuclei are unaffected, though bodies are found to be attached to them. In extreme cases, the nuclei present the appearance of crescent-shape, shrivelled up structures, recognized as such only by the presence of nucleoli. Nuclear degeneration in virus-affected cells has been observed in the case of vaccinia, where the bodies indent the nuclei. Kunkel (15, 16) also observed dented crescent-shape nuclei, in association with the bodies, found in the case of the Fiji disease of sugar cane and Hippeastrum mosaic. Goldstein (7), however, found that the nuclei were not affected by the bodies in the case of the tobacco mosaic.

THE NATURE OF THE BODIES

There has been much divergence of opinion in interpreting the nature of the intracellular bodies associated with plant and animal viruses. While some of the original investigators believed the bodies to be of the nature of organisms and classed them under the Chlamydozoa-Strongyloplasma group, later investigators have been inclined to the view that they are products of cell reaction produced by virous injury. Observations of movements in the bodies, and of the process of division, noted by Goldstein (7) and Likhité (17) in the cells of tobacco mosaic and by Kunkel (15) in the case of bodies associated with the Fiji disease of sugar cane, are evidences in favor of the view that they may be considered to be organisms. That the bodies consist of living cytoplasm, with chondriosomes in moderate numbers distributed in them, was established by Holmes (12) in the case of Hippeastrum mosaic. Henderson Smith (26), however, found that the inclusions, associated with *Solanum nodiflorum* infected with aucuba mosaic, were formed by the aggregation of small particles carried in the protoplasmic streaming and that they crystallize into forms that give a protein reaction. With our present knowledge, the intracellular inclusions associated with the spike disease of sandal, as in other viroses, can have only a pathognomic value.

SUMMARY

1. A cytological study of the spike disease of sandal was made with the result that intracellular bodies were found associated with this disease, similar to those described in other virous diseases.

2. Various staining reactions of the bodies have been tried. It was of interest to note that the bodies reacted in the same way as those found in animal virous diseases, such as rabies and epithelioma contagiosum, with Goodpasture's staining method, which, hitherto, has not been tried by workers on plant viruses.

3. Proliferation of the bodies was noted in some of the cells. The question whether the tiny bodies found in some of the cells are due to the proliferation of the large bodies is left open.

4. The effect of the disease on the cell contents was noted. The chloroplasts are stimulated to form an excess of starch. In the later stages of the disease, the disintegration of the chloroplasts sets in. When in association with the bodies, the flattened or dented appearance of the nuclei is of frequent occurrence.

MYSORE STATE,
BANGALORE, INDIA

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PHYTOPATHOLOGICAL NOTES

Dietrich Preferable Authority for Cronartium Ribicola.—Klebahn's[1] recent publication of Dietrich as the authority for *Cronartium ribicola* is of interest to Americans because the fungus is a permanent factor in North American forestry and because our extensive researches on the disease must be continued for an indefinite period. In 1909 the writer encountered the problem of the author who should be credited with the name *C. ribicola.* As it was impossible to find anywhere in North America one of the original specimens issued by Dietrich,[2] it was not known whether the label may have borne any sort of description that would entitle the name to a "legal" status. Arthur[3] stated that the name should be "*C. ribicola Fischer de Waldheim,*" which was used for some time. On mentioning the use of Fischer de Waldheim to Farlow several years later he said that he was sure that this was incorrect, but he was unable to ascertain which Fischer was responsible for the name. After that time the name was given as *Cronartium ribicola* Fischer in all of our publications. Still later, in a personal search in European herbaria, the writer failed to find one of Dietrich's specimens. He, thereupon, concluded that settling the authority for *C. ribicola* must await action of some European investigator who might find a specimen. Some time ago Klebahn wrote suggesting that Dietrich ought to be the author of *C. ribicola* and urging the writer to try to clear up the matter. Again, however, it seemed impossible to do more than already had been done, and pressure of other more insistent matters led to this one being laid aside.

In the meantime much space had been paved with good intentions and Klebahn[4] had answered the problem himself. His method of attack was as follows: Dietrich's name technically was a *nomen nudum,* but he showed plainly that the fungus was a Cronartium and lived on Ribes. He then considered Fischer's[5] name and concluded (justly the writer believes) that the fungus was inadequately described to distinguish it from other species of Cronartium, *except for the fact that it lived on Ribes.* He then took up more recent and complete descriptions, like those of Ed. Fischer[6] and his

[1] Klebahn, H. Der Autor des *Cronartium ribicola.* Ztschr. Pflanzenkr. 41: 209–213. 1931.

[2] Dietrich, H. A. Plantarum florae balticae cryptogamarum. Centuria 4, no. 21. 1855.

[3] Arthur, J. C. Uredinales. North American Flora 7: 122–123. 1907.

[4] See footnote 1.

[5] Fischer. *Cronartium ribicola* Fischer nov. sp. Rabenhorst. Fungi europaei exsiccati. no. 1595. 1872; Hedwigia 11: 182. 1872.

[6] Fischer, Ed. Die Uredineen der Schweiz. 590 pp. K. J. Wyss, Bern. 1904. pp. 433–435.

own.[7] He decided that all failed to distinguish *C. ribicola* from other species of Cronartium *unless the host were known.* Even then, on the same Ribes host, species like *C. ribicola* and *C. occidentale* can be distinguished only by biometric means or pedigreed cultures. He, therefore, concluded that, without knowing the pine hosts, Dietrich's simple statement that it was a Cronartium living on Ribes was as good as later descriptions and that he should be given credit for discovering, recognizing, and naming the fungus. The writer is heartily in sympathy with this decision, which he believes is a result of sound common sense and logic. Hereafter he intends to write the name *Cronartium ribicola* Dietrich.—PERLEY SPAULDING, Division of Forest Pathology, New Haven, Conn.

Comparison of Pycnial Stage of Cronartium Ribicola on Pinus Lambertiana and P. Monticola.—Studies were recently conducted at Daisy Lake, British Columbia,[1] to determine the relative susceptibility of western white pine, *Pinus monticola* D. Don, and sugar pine, *P. lambertiana* Doug., to white-pine blister rust (*Cronartium ribicola* Dietr.). One hundred and seventy-seven western white pines and 114 sugar pines were used for the test. These young trees ranged from about 6 in. to 2 ft. in height at the time they were planted, and some of them are now slightly over 5 ft. in height. Size of tree had no apparent influence on the production of the spore stages of the rust on either species.

During the course of the susceptibility studies a marked difference in pycnial production of the rust on the 2 hosts was noted. The following observations are based on 129 cankers on 42 infected western white pines and 179 cankers on 49 infected sugar pines.

On seedlings of western white pine the pycnia first began to appear about the latter part of May or the first part of June, reached their peak of production during July or August, and continued to develop generally until some time in October. They were produced in great abundance. The pycnial drops were very large and usually coalesced into continuous blotches or sheets. The pycnial scars were prominent rusty brown to bluish black spots.

On the sugar-pine seedlings pycnial production did not begin until some time in July or August and did not continue quite so late in the season as it did on western white pine. The pycnial drops were very small, usually widely separated and distinct from one another, and rarely coalesced. At

[7] Klebahn, H. Uredineae, in Kryptogamenflora der Mark Brandenburg. vol. 5a: 718–723. 1914.

[1] Lachmund, H. G., and J. R. Hansbrough. Preliminary report on the relative susceptibility of sugar pine and western white pine to blister rust. Jour. Forestry 30: 687–691. 1932.

no time during the summer were they observed on all cankers. The pycnial scars were generally indistinguishable and lacked color.

Generally, on western white pine the rust is prolific and abundant not only in pycnial production but also in aecial production. The aecial production on sugar pine was similar in this respect to that on western white pine, regardless of the meager amount of pycnial production.

The rust is not yet known to occur on sugar pine within its natural range. Consequently, the development and production of pycnia and aecia on this host as herein described cannot as yet be considered typical for the species. The production and development of pycnia and aecia on the western white-pine seedlings were typical of those observed on this species over a period of several years in numerous localities.—JAS. L. MIELKE, Division of Forest Pathology, Bureau of Plant Industry, Portland, Oregon.

A Correction.—In an article in PHYTOPATHOLOGY (A hitherto unreported disease of maize and beans. Phytopath. **22**: 637–644. 1932), a second article, by J. C. Haigh, (*Macrophomina phaseoli* (Maubl.) Ashby, and *Rhizoctonia bataticola* (Taub.) Butler. Ann. Roy. Bot. Gard., Peradeniya, **11**: 213–249. 1930), should have been included in the bibliography. The reference on page 641 to Haigh's inoculation experiments should relate to this article instead of the one cited. On page 642 reference to both articles should have been made in the following statement, "The pycnial stage, . . . found by Ashby and Haigh to occur in beans and sunflowers in Asia, . . ." Also, on page 642, the correct reference to Haigh's division of the sclerotial forms of *Rhizoctonia bataticola* into three classes is the 1930 article instead of the 1928 publication cited.—W. W. MACKIE, University of California, Berkeley, California.



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THE PINEAPPLE MEALY BUG, PSEUDOCOCCUS BREVIPES, AND WILT OF PINEAPPLES[1]

WALTER CARTER

(Accepted for Publication June 29, 1932)

INTRODUCTION

The first reference to wilt of pineapples in Hawaii was made by Larsen (15) in 1910. From his description and photographs it is evident that the condition was present in the islands at that time. Higgins (11, p. 30), in 1912, reported damage from wilt as limited to a few fields. Since the organization of experimental work on pineapples, numerous references to plant failure have been made in local records under the generic term "wilt." The problem evidently increased in importance, for by 1920, according to Illingworth (13), "whole fields were wiped out." By the end of the next decade, the wilt problem was recognized as the most serious with which growers had to deal.

It is evident from a consideration of the literature that the term wilt has been used in a generic sense to include many types of plant failure. Sideris and Paxton (17) have summarized the literature on root-rotting fungi with respect to plant failure and concluded that "Root rot caused by pythiaceous organisms is probably one of the important causes for the development of the disease of pineapple plants known as 'wilt' ". These same authors (16) also report pythiaceous organisms of heart rot as contributing to plant collapse under extremely localized and specific conditions of temperature and humidity. Their conclusions, however, as to the effect of root rot on the development of wilt are as follows: "Resistance in pineapples may be interpreted as the ability of infected plants to produce new roots and thus perpetuate the life of the plant and susceptibility as the converse of this reaction."

Illingworth (13) presented the first evidence that the pineapple mealy bug, *Pseudococcus brevipes* (Ckl.), was an important factor and concluded that wilt was a disease transmitted by mealy bugs that had fed upon diseased plants. He also considered that the green spotting of the leaves, which sometimes follows mealy-bug feeding, was a characteristic symptom

[1] Published with the approval of the Director as Technical Paper No. 47 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

of wilt that could be inherited from mother plants by the vegetative planting material.

In the present writer's investigations, described in this paper, the first step was to determine conclusively the relationship between *Pseudococcus brevipes* and pineapple wilt. In the course of such study other significant questions arose that are related to the principal problem. These were duly considered and the results are included in this paper. A concurrent study of the various types of spotting caused by mealy-bug feeding has been conducted and the results are being published elsewhere (5).

DESCRIPTION OF THE DISEASE

"PINEAPPLE WILT": "EDGE WILT"

Larsen (15) described the most characteristic symptom as loss of rigidity of the leaves. Color changes were noted as occurring but not without exception. Roots were invariably found dead and filled with fungus mycelia. His illustration is typical of what is now recognized as mealy-bug wilt. Illingworth (13) also described the disease, calling attention to color changes associated with loss of rigidity. He also mentioned the fact that, typically, wilt begins at the edges of fields or rock piles within field areas.

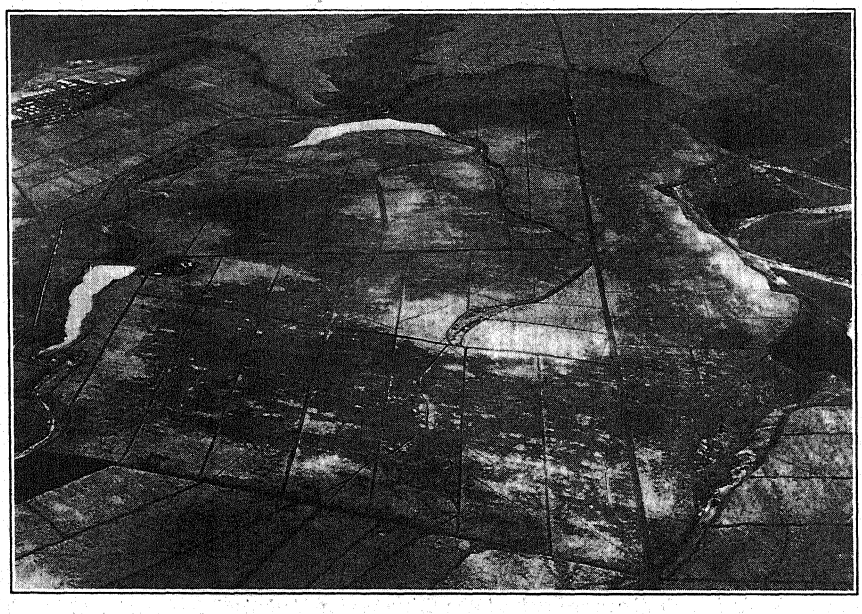


FIG. 1. Aerial photograph of pineapple field, showing distribution of wilt along field margins and intra-field ditches. Photographed by the 11th Photo Section A. C., Luke Field, Oahu, Territory of Hawaii.

This edge wilt, as plantation men came to call it, appeared to the writer, after numerous field observations, as a most significant concept. It suggested strongly the invasion of the field by some causal agent. Figure 1 is an aerial photograph of a field showing edge wilt in pronounced degree.

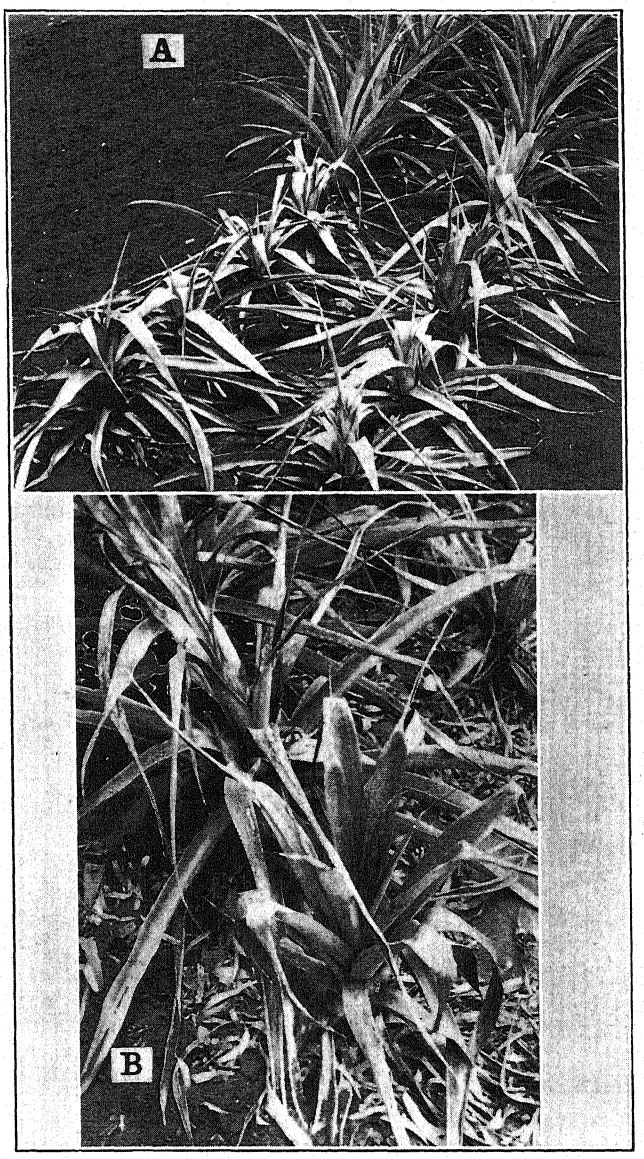


FIG. 2. A. Quick wilt produced in field plots. B. Slow wilt occurring after large colonies of mealy bugs had fed on plants for approximately 1 year.

It will be observed that the lighter areas, which represent areas of wilting plants, are concentrated either on the field edges or next to weedy drainage ditches.

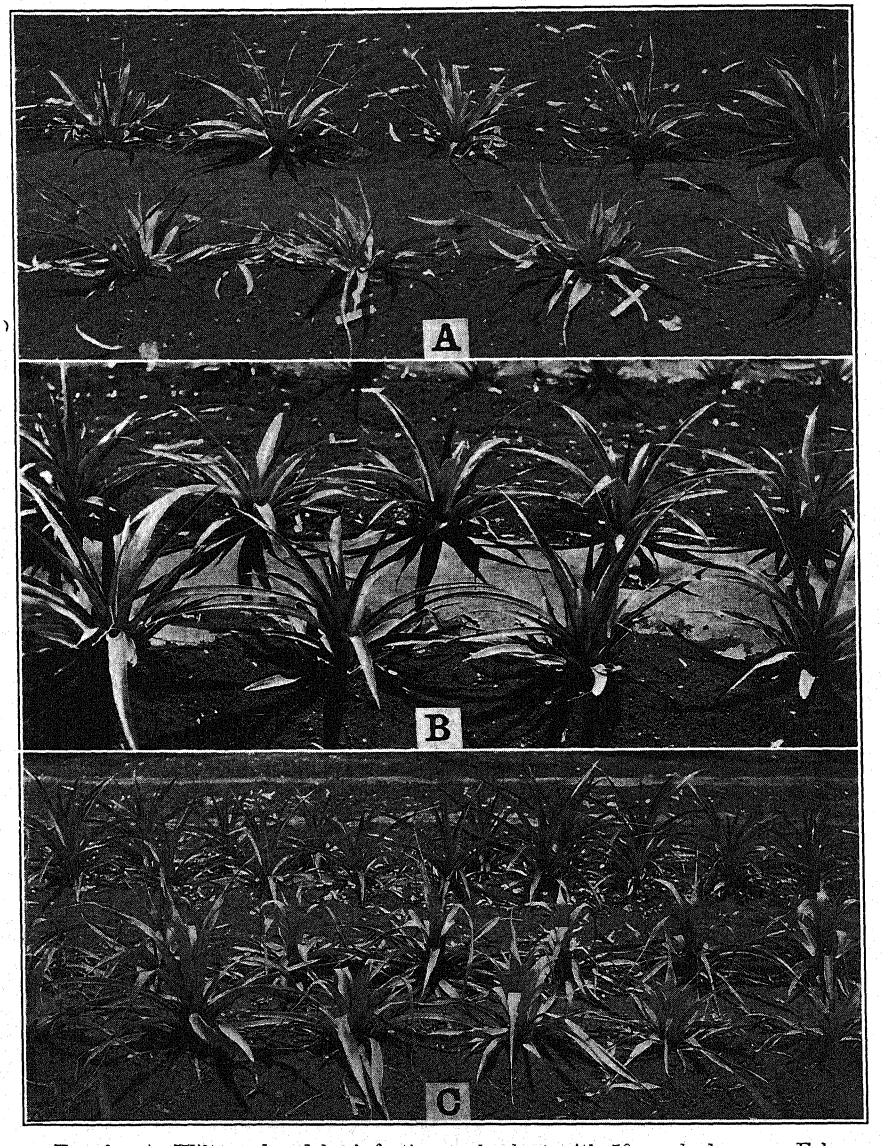


FIG. 3. A. Wilt produced by infesting each plant with 50 mealy bugs on February 3, 1931. Photographed April 30, 1931. B. Check plants of figure 3, A. C. In foreground plants infested with 50 mealy bugs per plant on April 27, 1931; in background check plot. Photographed July 6, 1931.

The following descriptions of the disease are based on plants used in experiments that are described in detail in this paper. In view of the terms used, the descriptions may be divided under the following headings:

QUICK WILT (FIGS. 2–5)

Occurs primarily in young plants. In plants up to 6 months old, the inner leaves become pale, varying from very light dull green to pale yellow

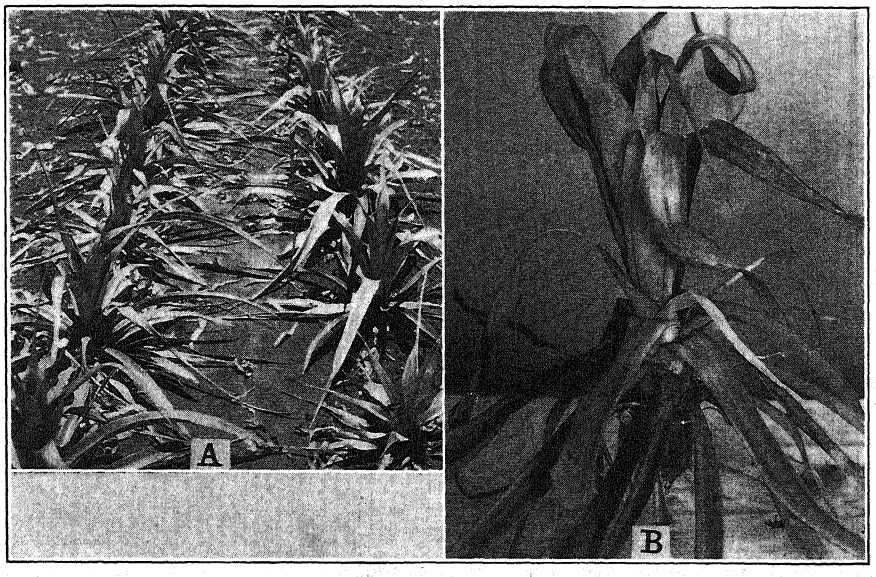


FIG. 4. A. Plot with quick wilt. B. Individual plant from plot shown in A. Photographed July 20, 1931.

or pink. With the color change is a very characteristic loss of rigidity, most of the inner leaves becoming flaccid and bending outward. The tips of these leaves turn brown and dry up. The inner leaves may show many or few or no green spots, or there may be scattered small chlorotic areas with irregular margins. With plants 8 to 10 months old, there is a conspicuous reddening of the leaves of the fourth or fifth whorl[2] from the center with a progression of color change to red, inward, to the center whorls. From that point color changes may occur either in the direction of bright pink and yellow or to a flat yellow brown. Development of the pink and red shade is controlled somewhat by the light-shade conditions. In dense plantings, the yellow and brown shades predominate. Secondary necrotic

[2] The term "whorl" is used for convenience in designating groups of 3 contiguous leaves in the normal spiral in which pineapple leaves grow.

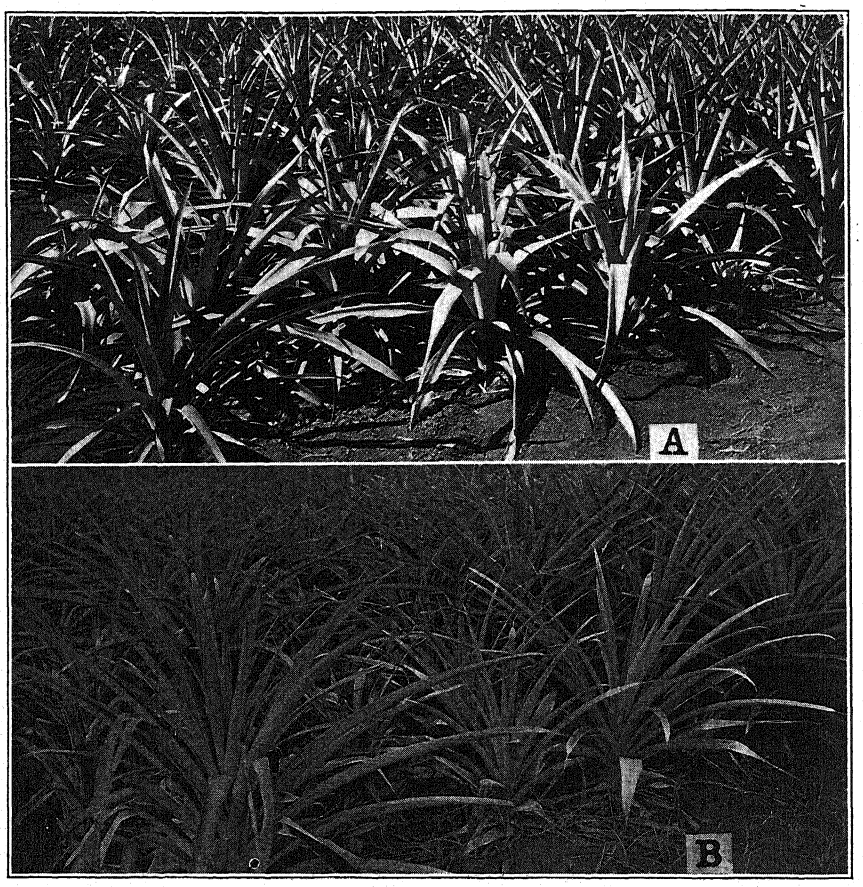


FIG. 5. A. Quick wilt in 4-line plot of Experiment III. Photographed July 20, 1931. B. Recovery in same plot. Photographed February 9, 1932.

areas appear on affected leaves, presumably the result of invasion of old feeding punctures by saprophytic organisms. When quick wilt occurs at early inflorescence or fruiting stages, the general appearance is one of dried up yellow brown leaves, frequently without the outward reflexing of the leaves. Quick wilt in young suckers and slips follows the same course as in very young mother plants. This type of wilt is produced by a short period of feeding by a fairly large colony of mealy bugs.

SLOW WILT

Wilt, occurring after the development of a large colony of mealy bugs, shows fewer color changes. A most characteristic symptom on slow-wilted plants is the large number of old mealy-bug feeding points. These may be of the green-spotting or chlorotic-spotting types (5). The leaves in this

type of wilt are usually so completely covered with these feeding areas as to eliminate most of the leaf tissue from functioning. The tips of these leaves are browned and dry and those of the outer leaves bend outward and droop, but there is none of the yellow pink characteristic of the quick-wilt type. The edges of the inner leaves are reflexed inward and flaccid to the touch but remain upright and retain a drab green color (Fig. 2, B). Secondary necrosis is commonly encountered.

In both types of wilt the roots are collapsed, invaded by saprophytic organisms, or dried up.

THE PINEAPPLE MEALY BUG

Pseudococcus brevipes is a member of the Homopterous family Coccidae. It has been reported (8) as partial to bromeliaceous and allied plants and is believed to be indigenous to Central and South America. In Hawaii it is found on a large number of host plants belonging to many families without close affinities. The principal hosts are pineapples, *Ananas comosus* (L.) Merr., banana, sisal, and several grass species, of which panicum grass, *Panicum barbinode,* and red-top Natal grass, *Tricholaena rosea* Nees, are the most important.

The insect is parthenogenetic and viviparous; its rate of development is slow, at least 2 months being required for development from newly emerged larva to gravid female; it is covered by a whitish secretion of wax and secretes honeydew; and it is almost invariably attended chiefly by two species of ants, *Pheidole megacephala* (Fabr.), and *Solenopsis geminata* Fabr. var. *rufa* Jerdon, respectively.

At the time of harvesting, the mealy bugs are found in large numbers on the fruit and on the slips and shoots that grow out from the fruit and mother stems. Damage to fruit has been recognized for some time (9) and its quantitative estimation determined recently (6).

The inception of infestation and its development and spread in pineapples have been studied and data presented in another paper (4). The movement into fields from wild hosts is characteristic and significant. Populations are thus established, first on the edge of fields with a gradual movement into the field area.

When these facts were established, the possible causal connection between edge wilt and the invasion of the field edges by mealy bugs was considered. Experimental work began with tests of *Pseudococcus brevipes* as a single factor in the production of wilt.

Experiment I. Pseudococcus brevipes as a single factor in the production of pineapple wilt

The planting material used for this experiment was obtained from a field that had just previously had its first or "plant-crop" fruit removed.

At this time the old fruit stem bears a number of "slips," which, when broken off and cured by drying, are used to plant new fields. The field from which these slips were taken was infested with mealy bugs and all the

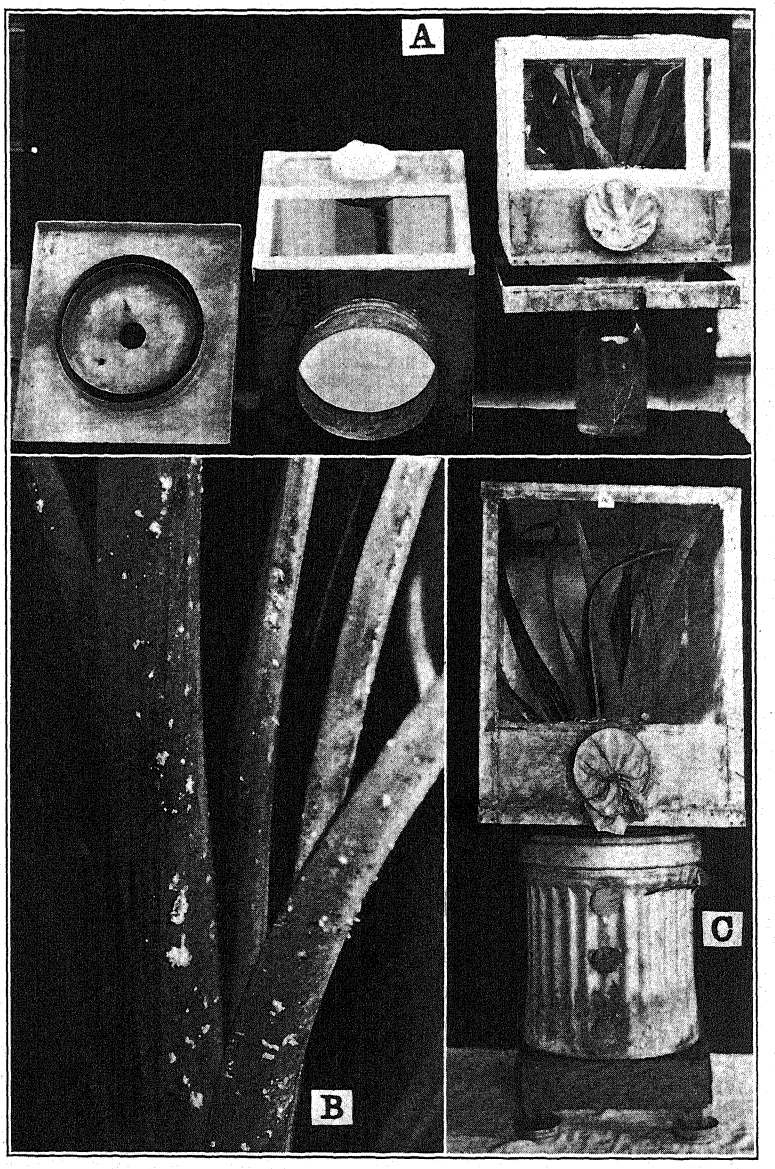


FIG. 6. A. Cage used for protecting pineapple plants growing in culture solution from accidental insect infestation. B. Mealy-bug colony unattended by ants. C. Cage used over pineapple plant growing in soil.

plants were green-spotted. Seven plants were stripped of their slips and each lot constituted a separately marked family. All were fumigated with hydrocyanic acid, as described by Hagan (10). These slips were then set in culture solutions under cages designed to exclude all but the experimental insects (Fig. 6, A). The slips from 1 family were planted in sterilized soil under somewhat similar cages (Fig. 6, C). Forty-four slips from the 7 parents were used: 20 were checks of which 3 developed small mealybug populations, due to inadequate fumigation; 2 were infested with 1 gravid female, each; 7 with 10 mealy bugs of various ages and 15 with 50 mealy bugs, all of which came from a badly wilted pineapple field. The infestation time was varied, being made on 14 plants in September, a month after planting, and on 10 in November, 1930. The results of this experiment are presented in summarized form in table 1. The notes under "Appearance of plants" cover the whole period of the experiment, which lasted for approximately 10 months.

TABLE 1.—*Pseudococcus brevipes as a single factor in the production of pineapple wilt*

No. of plants	No. of insects applied to each plant	Appearance of plants	Green spotting	
			On old leaves	On new leaves
2	1	Normal	++	++
4	10	"	++++	+++[a]
2	10	No typical symptoms	++	++
1	10	Partial wilt	+	+
9	50	Normal	On all	On all
1	50	No typical symptoms		
4	50	Partial wilt	+++	+++
13	None	Normal	On all	On 3[b]
6	None	No typical symptoms	++++	----

[a] Mealy bugs failed to establish.

[b] Small populations of mealy bugs developed.

Discussion of Experiment I. From the above table it is seen that new growth became green-spotted on all but the 1 plant on which populations failed to develop. Typical wilt symptoms developed in 2½ months on 1 plant and in a less pronounced manner on 5 others in from 4 to 6 months. The most typical case of wilt obtained in this experiment (Fig. 7, A) was removed from its cage and dissected. The green spots on the leaves, evi-

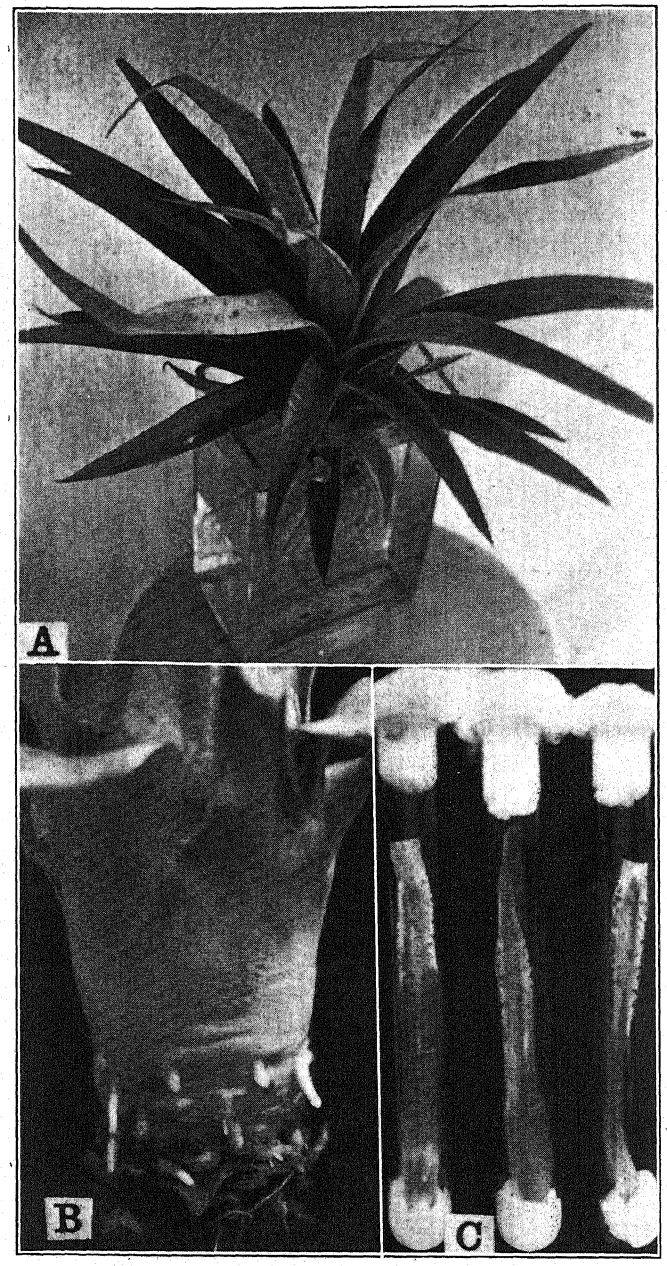


FIG. 7. A. Plant grown in cage (Fig. 6, A), showing typical wilt symptoms after infestation with mealy bugs. B. Plant of figure 4, B, 1 week after being trimmed and set in culture solution. Note new roots. C. Leaves from wilted plant used for tests on the effect of host sequence on toxicity of *Pseudococcus brevipes*.

dence of mealy-bug feeding, were counted for each whorl of leaves. These data are listed below, the whorls being numbered, beginning at the inside growing point of the plant.

Whorl 1. Only tips visible and they were necrotic. *Whorl 2.* First and 2nd leaves pink and flabby; 3rd leaf still green, except for browned tip: all covered with green spots. *Whorl 3.* All pink and flabby: covered with green spots. *Whorl 4.* First leaf—54 spots on new growth, which was green. 2nd leaf 169 spots: leaf pink at tip and flabby. 3rd leaf pink and flabby, covered with spots. *Whorl 5.* Two, 20 and 38 spots on new growth of leaves, normal except for slight paling. *Whorls 6 to 10.* Perfectly green and normal: scattered green spots on old growth; none on new.

From this case it appeared that a relationship existed between area actually fed upon and amount of tissue collapsed. Five other plants showing localized wilt indicated further the possible quantitative relationship between area actually fed upon and leaf collapse, the area of leaf collapsed being coincident with that heavily spotted. Some of these cases simulated a type of wilt frequently found in the field and designated as "terminal" wilt. In this type the ends of the median whorls of leaves are pink, often with sharp demarcation between normal and affected tissue, while the center whorls are normal. This indicated that such types were the result of a short-lived mealy-bug colony's feeding, which damaged tissue locally without any appearance of systemic effect. Some plants of this experiment showed green spotting on all the leaves that developed after infestation, for a period of 6 and 8 months, unaccompanied by any wilt symptoms. This indicated that green spotting *per se* was not a concomitant of wilt; neither did green spotting on planting material forecast later wilt of the plant. It will be noted that on only one of the 9 plants infested with less than 50 mealy bugs did typical symptoms develop and then only in a mild way, even though all these plants showed typical green spotting.

This last question was tested separately by growing a series of plants that had been so heavily infested as to leave the foliage covered with green spots. The material was fumigated to remove mealy bugs and grown in isolation cages. New growth was spot-free and perfectly normal in color, vigor, and growth. As was true in some of the plants of Experiment I, the old heavily spotted basal leaves yellowed and dried down.

Effect of absence of ants. It usually has been considered that the protective influence of ants was the major contribution to the mutual relationship of ants to mealy bugs. Experiment I, however, indicated clearly that a mealy-bug colony unattended by ants was abnormal. Masses of honeydew and cast skins accumulate and the distribution of the young is seriously impeded. Instead of remaining in the heart of the plant where the most succulent tissue is, these small masses of gummy mealy bugs remain

on a very small area of leaf as the leaf grows out, sometimes persisting even when the leaf has become shrivelled and brown (Fig. 6, B). It is significant in this connection that the most typical case of wilt obtained was one in which the mealy bugs promptly established themselves in the heart tissue.

This experiment established for the first time the rôle of *Pseudococcus brevipes* as a single and primary factor in the production of typical pineapple wilt. The low incidence was attributed to abnormal mealy-bug colonies, but the influence of the field complex was also considered, and an experiment was set out in the field plots. Progress in mealy-bug control gave some assurance that check plots could be kept clean by spraying (3).

Experiment II. Effect of adding mealy bugs to pineapple plants, growing under natural field conditions

This experiment considered 3 possible variables: size of initial infestation, time of infestation, and type of planting material. There were 2 cases in each class, as shown in table 2.

TABLE 2.—*Arrangement of Experiment II*

Planted	Infestation dates			
9/25/30	2/3/31		4/27/31	
Good planting material	1 gravid female per plant	50 mealy bugs, mixed sizes and ages	1 gravid female per plant	50 mealy bugs, mixed sizes and ages
Poor planting material	1 gravid female per plant	50 mealy bugs, mixed sizes and ages	1 gravid female per plant	50 mealy bugs, mixed sizes and ages

The selection of good and poor planting material was necessarily empirical, but the growth of the 2 types showed the expected differences in uniformity and vigor. There were 50 plots in all, containing 1,000 plants, of which 400 were scattered in 20 check plots. Each plot consisted of a section of a regular 2-line bed and contained 20 plants. This set-up was duplicated in another field, but the 2 areas showed essentially similar results. The better planting-material plots showed uniform growth; the poorer planting material was uneven, some being very slow in starting. These differences became less pronounced as the season progressed. The mealy bugs used in these tests were obtained from wilting plants and came from an area where green spotting was general.

There was rapid establishment of the colonies with noticeable differences obtaining, however, between the establishment on the plots planted with good planting material and some of the plants in the plots of poor planting

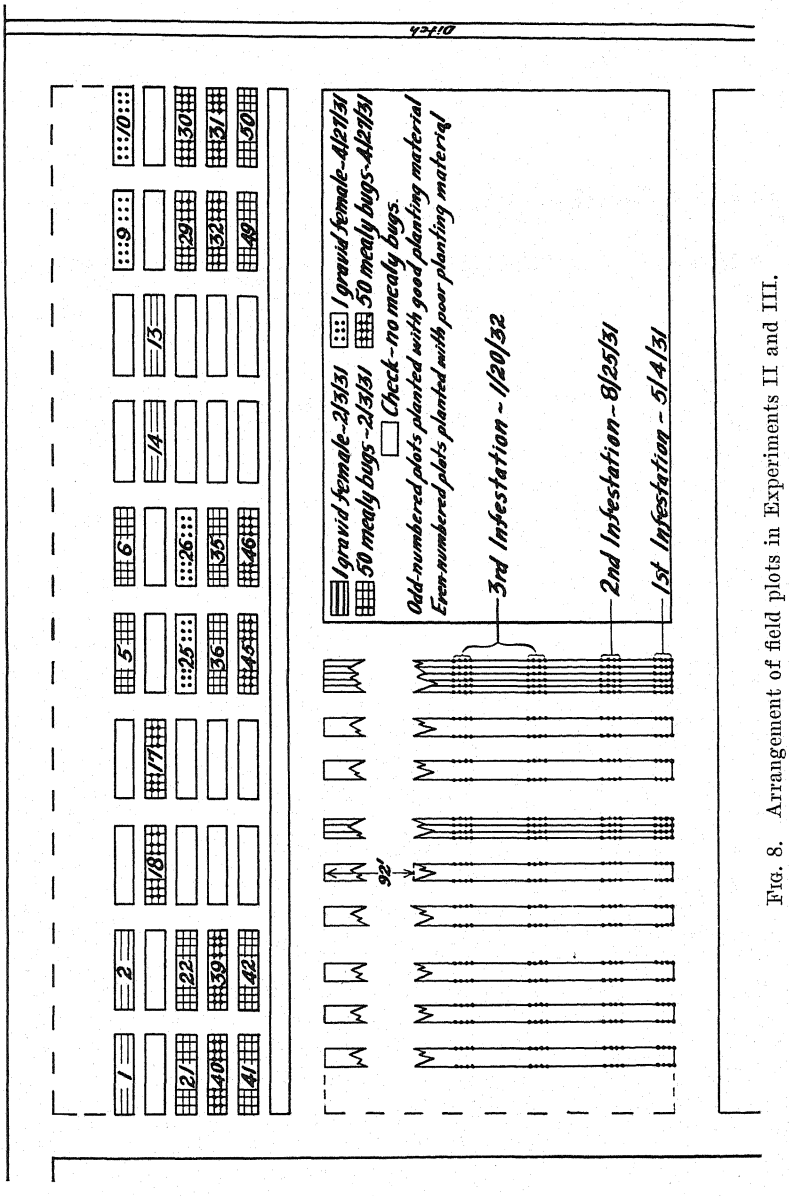


FIG. 8. Arrangement of field plots in Experiments II and III.

material. The former, being uniformly succulent and vigorous, showed uniformly rapid establishment of the mealy-bug colonies; the latter included some individuals that were retarded, and these had tougher tissue. Establishment on these plants was noticeably less rapid. In the area included in the experimental plots, Pheidole ants were present and promptly attended the mealy-bug colonies put on the plants. In figure 8 is shown the arrangement of plots for this and a subsequent experiment, and the results of the experiment are presented in tables 3 to 5.

The results of this experiment, besides demonstrating the importance of *Pseudococcus brevipes* in producing wilt under field conditions, revealed something of the intricacy of the problem and gave some suggestions as to the nature of the injury.

The response of the plants to the infestation of 50 mealy bugs made on February 3, 1931, when the plants were 4 months old, was quite clear. Typical flabby collapse was evident April 27, 1931, on a considerable number and by July 6, 1931, the whole series showed almost complete collapse (Fig. 3, A and B). The mealy-bug populations were at this time extremely small, evidence of the fact that the bugs will desert a wilting plant.

The response to single mealy-bug-infestation experiments made on the same date was, however, quite different. These plants had, in the course of time, developed large populations of mealy bugs; yet the only evidence of plant response, except in the case of 5 out of the 80 plants in these plots, was in a slight spindliness of growth. These populations continued to increase until the basal portions of the inner leaves were literally covered by the colony. There was some variation in size of these colonies between 2 widely separated pairs of plots, which was of some significance in the end result. All these single individual infestations, however, developed populations far in excess of those responsible for nearly complete collapse in the case of plants that were subject to an initial population of 50 mealy bugs on the same day.

On April 27 the second series of plots was infested. Plots that, up to that time, had been kept clean of mealy bugs by means of sprays were infested with 1 gravid female per plant or 50 mealy bugs per plant, as in the first series. As before, Pheidole ants promptly appeared in attendance. The plants by that time, of course, were larger and more succulent than at the time the first series was infested. All were normal in color and growth. The results of this second infestation added more evidence as to the ability of *Pseudococcus brevipes* to produce a wilted condition of the pineapple plant, for, on July 6, approximately 10 weeks after infestation, some of the heavily infested plots showed complete collapse and all showed a high percentage of wilted plants (Fig. 3, C). The plants infested with 1 female, apart from developing considerable populations of mealy bugs during that time, showed no typical symptoms, except in 1 case.

TABLE 3.—*Wilt occurring after 1 gravid female mealy bug had been applied to each plant. Twenty plants in each plot. One series of plots infested February 3, 1931, and another on April 27, 1931*

Plots infested Feb. 3	Total number of plants wilted in each plot on following dates					Condition of plants as observed April 11, 1932			
	4/27/31	7/6/31	9/18/31	11/11/31	2/9/32	No recovery	Wilted in fruit stage	New leaves normal in color but spindly	Normal but small fruits
1 G	1	1	2	10	18	20	0	0	0
2 P	0	2	4	9	18	11	1	5	3
13 G	1	2	0	5	4	4	5	0	11
14 P	0	0	0	1	6	7	3	8	2
Plots infested April 27					2/3/32				
9 G	0	1[a]	2	—	3	9	0	0	11
10 P	0	1[a]	0	—	1	4	2	6	8
25 G	0	3[a]	—	10	9	4	5	3	8
26 P	0	2[a]	—	2	2	0	2	5	13

[a] With the exception of 1 of the plants of Plot 26, none of these plants showed typical symptoms but only reddening of the *outer* leaves.

TABLE 4.—*Wilt and recovery occurring after 50 mealy bugs had been applied to each plant. Twenty plants to each plot. Plots infested Feb. 3, when plants were 3 months old*

	April 27, 1931			July 6, 1931			Feb. 9, 1932			
Plot	Typical wilt	Plants retarded only	Plants normal	Plants apparently dead	Plants abnormal but still growing	Plants normal	No recovery	New growth normal in color but spindly	Recovery of terminal wilt type	Normal but retarded
5 G	16	3	1	17	2	1[a]	1[a]	19	0	0
6 P	7	5	8	12	8	0	0	14	0	6
21 G	20	0	0	14	6	0	1	19	0	0
22 P	10	0	10	12	6	2	3	15	0	2
1 G	3	0	17	17	2	1	0	11	9	0
2 P	2	0	18	14	6	0	0	14	0	6
9 G	19	0	1	20	0	0	0	7	13	0
10 P	10	0	10	14	0	6	3	13	0	4
15 G	1	0	19	14	3	3	0	3	10	7
16 P	0	0	20	5	14	1	1	0	4	15

[a] This plant is shown in figure 12 A and B.

TABLE 5.—*Wilt and recovery occurring after 50 mealy bugs had been applied to each plant. Twenty plants in each plot. Plots infested April 27 when plants were 5 months old*

Plot	April 27, 1931			July 6, 1931			Feb. 9, 1932		
	Typical wilt	Plants abnormal	Plants normal	Typical wilt	Plants abnormal but still growing	Plants normal	No recovery	New growth normal in color but spindly	Normal but retarded
17 G	1	6	13	14	6	0	0	20	0
18 P	0	0	20[a]	9	5	6	5	15	0
29 G	0	0	20	19	0	1	0	20	0
30 P	0	0	20	7	10	3	1	19	0
5 G	0	0	20	13	0	7	0	19	1
6 P	0	0	20[a]	16	0	4	3	10	7
11 G	0	1	19	19	0	1	0	20	0
12 P	0	0	20[a]	11	0	9	1	17	3
19 G	0	0	20[a]	16	0	4	2	18	0
20 P	0	0	20[a]	14	0	6	2	18	0

[a] Plants uneven in growth.

It will be observed that the plants infested with 1 gravid female showed little signs of collapse in any of the plots until November, 1931. In February, 1932, plants in plots 1 and 2 were nearly all wilted, while plots 13 and 14 still showed a low incidence. This difference was associated with great differences in populations. Counts made prior to collapse in plots 1 and 2 revealed that plots 1 and 2 were carrying populations of approximately 1,500 mealy bugs per plant, while populations on plots 13 and 14 were approximately 500 mealy bugs per plant. Observations made in April, 1932, showed plots 13 and 14 still superior to plots 1 and 2 in amount of recovery and numbers of normal plants.

Comparison of the plots planted to good and poor planting material and both heavily infested indicates what was obviously true on inspection, that the more vigorous succulent growth was more susceptible to wilt produced by a sudden attack of mealy bugs.

Plots 5 and 6 present additional data on this point. It will be observed that 8 plants were still growing on July 6, 1931. These plants had been noted as retarded and with poor establishment of the mealy bugs added 2 weeks after infestation. They continued to grow and, though somewhat smaller than the lightly infested plants, were, nevertheless, normal in color and growth 8 months later, by which time very large populations of mealy bugs had developed. In April, 1932, most of these plants were still normal in appearance except for retardation and evidence of heavy mealy-bug feeding on the leaves. The capacity of the plants to withstand a high population of mealy bugs over a long period of time, if the population develops from a low initial infestation, was strikingly illustrated in this experiment. On the other hand, the high toxicity of a large initial dose demonstrated beyond question the capacity of *Pseudococcus brevipes* to produce typical wilt of pineapple plants under field conditions.

In all these cases of wilt, in both light and heavy infestations, green spotting was pronounced and, in the case of the plants collapsing after a period of a year, following extremely heavy feeding, the leaves were covered with spots in various stages of development, from freshly made zonate spots to welts and areas of secondary necrosis.[3]

The behavior of the check plots was carefully observed, since, from them, suggestions as to other inimical factors could be obtained. There were 20 check plots in all, each containing 20 plants. In addition, up to the time of making the April 27, 1931, infestation, all those plots were also checks and at that time a few scattered plants showed symptoms of abnormality. This usually expressed itself as a reddening of the *outer* older leaves of the plant. Of the 400 plants in the checks on July 6, this redden-

[3] The descriptions of the types of green spotting and their development are too long to be included in this paper. They are considered more fully in another manuscript (5).

ing of the outer leaves was somewhat evident on 18, 10 of which were in one of the plots planted to the better grade of planting material. Samples of these were submitted to the Pathology Department for examination for pathogens, with negative results.

Six of the 400 were typically wilted, a condition that could not be ascribed to any known mealy-bug establishment, since these plots had been sprayed with oil emulsion at approximately monthly intervals. These 6 cases and others that followed were inexplicable until the data of the next described experiment were obtained, which indicated that these cases were the result of movement of mealy bugs from adjacent heavily infested plots. Observations made on August 18, 1931, revealed that all these cases were in direct apposition to wilted plots from which the mealy bugs had migrated.

The causality of the reddened outer-leaf condition remains unknown. As a factor, its low incidence and the fact that all the plants so affected grew normally after a short period of retardation suggest that its contribution to the collapse obtained in the infested plots was slight.

While the laboratory experiment (No. I) indicated the minor position of green spotting, with respect to wilt, the generally spotted condition of the wilted plants in this field experiment left the question a moot one as far as quick wilt was concerned. The next experiment recorded additional data on this question and, at the same time, revealed a previously unsuspected phase of the wilt problem.

On April 27, when the second series of plots in Experiment II was infested, an adjoining plot appeared to offer an opportunity to test the reaction of much larger plants under several conditions of planting. This plot had been laid out in 2-, 4- and 6-line beds as per figure 8. At the time of infestation, May 4, 1931, these plants were vigorous and normal in every respect. This plot was in the same field as Experiment II and planted at right angles to those plots, so that one end of each bed was contiguous.

Experiment III. Response of pineapple plants growing in various densities of planting to an initial infestation of 50 Pseudococcus brevipes

The inner ends were protected from infestation by virtue of their distance from infested plants, so the last 4 plants in each line of 9 beds were infested with 50 mealy bugs taken from wilting plants. At the time the 50 bugs were applied, these lines were free from ants. As frequently happens in the field, absence of ants means either poor establishment or dissolution of the mealy-bug colony. The infestations applied on May 4, 1931, did not establish and increase. The toxic dosage applied to these plants, therefore, was that imparted by the original 50 mealy bugs by feeding for a short time.

Observations made the latter part of June showed many of these plants "off color," that is, with the inner whorls of leaves distinctly reddened, and on July 6 a striking effect of the mealy-bug infestation was definitely evident. There was almost no evidence of movement of mealy bugs from the infested plants to the adjacent plants in the same lines except in 1 case where the 5th plant was infested and exhibited typical symptoms of a milder type (Fig. 2, A). The clean-cut division between infested and noninfested plants was so clear as to remove any remaining doubt as to the rôle of *Pseudococcus brevipes* in the production of wilt. Even more striking was the fact that the infested plants showed no evidence of mealy-bug feeding (*i.e.*, green spots) except in isolated cases, and, even in these, very careful examination was necessary to find them. In other words, a condition that had, in the past, been ascribed to a complex of root troubles because of the fact that no insect damage or insects could be found at the time symptoms were evident was here shown to be a phase of mealy-bug wilt. The symptoms of wilt in these cases were identical with those of Experiment II, except for the fact that green spotting was either extremely rare or absent. Table 6 summarizes the results of this experiment.

Relation of Age of Plant to Susceptibility to Quick Wilt. On August 25, 1931, the wilt in the foregoing experiment was still localized to the infested ends of each bed, the balance being vigorous and normal. A second section

TABLE 6.—*Response of pineapple plant growing in various densities of planting to an initial infestation of 50 Pseudococcus brevipes: Effect of age of plant on susceptibility.*

First section infested 5/4/31 Plants 6 months old		No. of plants collapsed		Notes on recovery			
Bed	No. of plants	7/6/31	10/2/31	February 16, 1932		April 11, 1932	
				Normal	Recovery	Normal	Recovery
2 line	8	8[a]	8[a]	0	5	0[b]	5
2 "	8	5	7	1	7	0	7 (1)[c]
2 "	8	4	5	3	5	3	5
2 "	8	6	8	1	7	1	6
2 "	8	4	5	3	5	2	6 (1)
4 "	16	12	13[a]	5	9	4	10 (1)
2 "	8	5	5[a]	2	5	2	5 (1)
2 "	8	5	7	2	5	1	6 (3)
6 "	24	14	20[a]	6	18	2	19 (6)

[a] Movement of bugs had occurred to 5th plant on one line. This plant, wilting.

[b] Fifth plant on one line now has small but normal fruit. All normal plants, this date, were fruiting.

[c] Figure in parentheses indicates number of recovered plants bearing small fruits.

TABLE 6.—(*Continued*)

Second section[a] infested 8/25/31 Plants 9½ months old		No. of plants collapsed			Notes on recovery	
					4/11/32	
Bed	No of plants	11/10/31	1/4/32	2/9/32	Wilted in early fruiting	Normal fruiting
2 line	8	2	4	4	3	4
2 "	8	3	2	3	3	5
2 "	8	3	7	6	5	1
2 "	8	6	5	5	3	4
2 "	8	3	2	2	1	7
4 "	16	10	10	12	11	5
2 "	8	8	8	8	8	0
2 "	8	4	7	7	7	1
6 "	24	9	12	14	11	12

[a] Third section infested 1/20/32—2 plots to each bed. Plants 14½ months old. 4/11/32 only one plant showing symptoms; it collapsed.

was then staked out in each of the beds (Fig. 8), consisting, as before, of 4 plants in each line. The plants at this time were 9½ months old. Mealy bugs from a wilting field were applied to these plants at the rate of 50 to each plant. At this time Pheidole ants were present and the mealy bugs established themselves.

The results of this second infestation are presented in table 7. There was considerable variation between beds in the amount of collapse ensuing and, on the whole, much less than occurred in the section infested May 4, when the plants were 3½ months younger. The reduced amount of collapse in the section infested August 25 was in spite of the fact that mealy-bug colonies established in that section and in April, 1932, were to be found in large numbers on the fruits. It was thus made evident again that the establishment of the colony was not necessary to ensure quick wilt, for in the first series practically complete collapse was obtained without establishment of colonies, while in the second series all the plants, not quick-wilting, developed large populations of mealy bugs.

The development of wilt in these last plots demonstrated that plants showing unmistakable symptoms of wilt prior to the appearance of the inflorescence could develop small inflorescences, which, however, wilted and dried up. This, a common occurrence in the field, was thus shown to be due to an abortive attempt at recovery by previously wilted plants. On the other hand, cases were obtained of this general type that represented the first appearance of wilt symptoms. In rare cases, recovery proceeded

to such an extent as to permit the development of a small but otherwise normal fruit.

Additional plots were marked out in the same beds (Fig. 8) and infested January 20, 1932, when the plants were 14½ months old. At this time the plants were in the stage just prior to the appearance of the inflorescence. The plots were duplicated in the same beds. Mealy bugs were taken from plants going into first ratoon[4] and showing symptoms of wilt. Establishment of colonies proceeded, but on April 11 only one plant was wilted.

Small colonies were to be found on the fruits at this time, but very much smaller than might have been expected from the size of the initial infestation and the length of time elapsing. The fact that colonies established themselves immediately after infestation, however, indicates that the plants were adequately exposed. Since only 1 case out of the 192 showed symptoms, it is evident that susceptibility to quick wilt decreases very rapidly with age of plant.

Quick Wilt not the Result of Local Injury. Results of Experiment III could not be explained on the basis of localized injury and suggested an extensive diffusion of an extremely toxic material. Proof of this was obtained when typical quick wilt resulted from the caging of mealy bugs on localized areas of leaves, the insects being placed as far down on the leaves as was possible without mechanical injury to the plant.

Comparison of the Toxicity of Mealy Bugs taken from Various Sources. The host-plant sequence of an insect is, no doubt, of importance in conditioning the effect of the insect on its host plant. This question is of general significance, for most, if not all, insect-transmitted or induced diseases of plants involve the movement or migration of the insects concerned from an alternate host or through a succession of hosts to the plant attacked.

It is true that with virous diseases the case is complicated by the reaction of the virus *per se* to the changed host conditions as well as that of the insect vector. Attenuation of the virus of curly top (1, 14) is probably primarily an instance of the former. A suggestion as to the possibility of the latter was made by Carter (2, p. 82). In the case of insects whose secretions are toxic as, for example, the leaf hoppers causing tip burn of plants, the probability of host sequence affecting the nature of the secretion of the insect is much greater and its demonstration involves less difficulty.

Experiment IV. Toxicity of mealy bugs as taken from the roots of 2 species of grass and from pineapple plants of various ages and conditions

In Experiment IV, mealy bugs were taken from 6 different sources as listed below:

[4] The suckers arising from the mother plant after the first fruit has been harvested form the plants of the first ratoon; those arising from the first ratoon plants, the second ratoon, and so on.

1. Red-top Natal grass, *Tricholaena rosea* Nees, growing along the edge of a pineapple field that had become infested from that source.
2. Healthy pineapple plants, growing a few feet away from the red-top grass. Green spotting was either rare or entirely absent from these plants. In the general area involved in both this and the following sample, high populations and wilt without green spotting was the general rule. The mealy-bug colonies here clearly represented edge infestation, as extensive sampling revealed its localization to that area of the field immediately contiguous to the grassy edge. It was difficult to find sufficient plants for this class, since remoteness from wilting plants was a desirable factor to include, and it was necessary to consider this factor in terms of a few feet.
3. Pineapple plants, showing initial symptoms of wilt. These were scattered along the edge of the field, rarely more than a few feet from the edge. It should be carefully borne in mind that the above 3 types had, according to all evidence, a common source of mealy bugs in the red-top Natal grass.
4. Panicum grass, *Panicum barbinode,* growing as an intercycle crop on a previously badly wilted field.
5. Young suckers of a coming first-ratoon pineapple field. These were taken from an area that had been under observation for some time and had contained high populations of mealy bugs the preceding year. There had been considerable collapse among the mother plants in this area and the suckers from which mealy bugs were obtained that were taken off for this experiment were all showing initial symptoms of wilt. Green spotting also was marked.
6. Small fruits and fruit tops, growing in an abandoned first ratoon field 1 year older than class 5. Tops were green-spotted. This was a typical field, abandoned because of wilt; most of the plants were wilted and the few remaining were characterized by tough tissue and had small stunted fruits. Considering the colonies obtained, from the standpoint of their nutritional history, there was probably considerable variation among them.

Using the above described insect material, 2 widely separated localities were chosen and plots arranged as per figure 9. One series replicated each treatment 3 times; the other, 6. Fifty mealy bugs were applied in September, 1931, to each plant in each plot, which included 20 plants, making a total of 1,080 plants in the 54 plots.

The results of this experiment are listed in tables 7 and 8.

Though the infestation conditions were exactly the same in these 2 locations, as far as initial infestations were concerned, the progress of the colonies differed materially. Location 1 was situated within a field a consider-

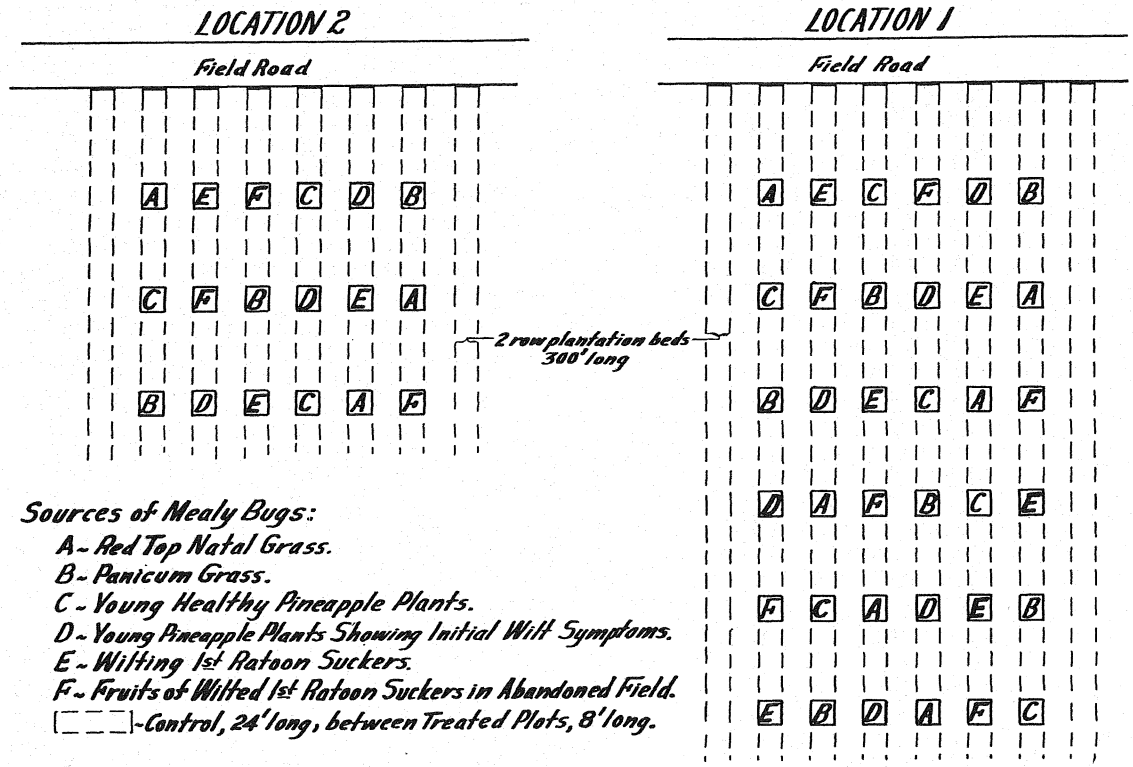


FIG. 9. Arrangement of plots in Experiment IV.

TABLE 7.—*Toxicity of mealy bugs from various sources. Plants infested during September, 1931. Total number of plants wilted in each series, at each date of observation*

Source	Location 1			Location 2		
	11/25/31	1/4/32	2/3/32	11/25/31	1/4/32	2/3/32
Red-top grass	0	6	6	2	3	5
Healthy pineapple plants contiguous to grass	2	4	11	2	6	11
Wilting pineapple plants from same area	3	53	66	6	30	41
Panicum grass	1	5	5	2	2	4
Wilting suckers from field going into first ratoon	24	73	80	21	51	53
First-ratoon fruits and tops from abandoned field	10	51	68	10	16	25

able distance from the edge. There were no ants in the vicinity. In addition to this, large numbers of *Conocephalus saltator* (Sauss.), a grasshopper reported (12) as occasionally predacious on mealy bugs, were seen in the plot. Examination of these plots the day after infestation showed establishment to be proceeding normally, but, prior to the first wilt count, careful examination revealed not a single colony established. In the other plots Pheidole ants appeared promptly after the mealy bugs were introduced. The 2 areas can thus be compared with respect to the influence of establishment of colonies on development of quick wilt. Location 1, containing twice as many plots as location 2, which was ant-attended, should have shown twice as much collapse if the establishment of colonies was entirely immaterial, a condition that did not hold true except in 1 case. The probability of extremely short-lived colonies in location 1 was great, and the results indicate that even quick wilt has a minimum dosage requirement.

From figure 10 it is seen that there was a gradual rise in toxicity, according to the source of mealy bugs. This toxicity reaches its peak in mealy bugs from young suckers from plant-crop mother plants. The reduced toxicity of mealy bugs from the ratoon crop, which was 1 year older, is possibly of some significance. If relative toxicity can be expressed in terms of time ensuing before collapse occurs, the mealy bugs from young

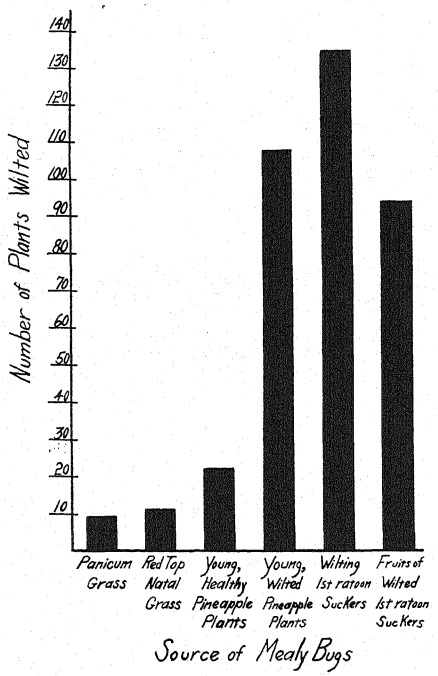


FIG. 10. Plants wilted when mealy bugs from various sources were used.

suckers were the most toxic in this respect also, since, on January 4, nearly all the collapse to occur took place in these plots.

Variability in the plots infested from any one source is of interest. This is shown in table 8, where individual plot totals are shown.

TABLE 8.—*Variation between plots infested with mealy bugs from the same source. Number of plants wilted in each plot 2/3/32, 5 months after infestation*

Source	Location 1						Location 2			Total of 9 plots
Panicum grass	0	0	1	1	1	2	0	1	3	9
Red-top grass	0	0	0	1	1	4	0	1	4	11
Healthy, young pineapple plants	0	0	1	2	2	6	2	3	6	22
Wilting, young pineapple plants	4	6	10	13	14	19	10	11	20	107
Wilting suckers	7	9	12	15	17	20	16	17	20	133
First-ratoon fruits and tops	6	7	10	12	13	20	6	8	11	93

These variations may be due to differences in susceptibility of individual plants. They may, however, be due rather to variations in the original mealy-bug infestations. The healthy plants used may have been previously infested for varying lengths of time and more or less close to showing wilt symptoms. The same holds with respect to the young plants in early wilt, since these differed considerably in the extent of evident wilting. The mealy bugs from young, wilting suckers were probably a more uniform sample than any of the others as to length of time spent by the colony on pineapples and also as to the stage of wilt developed in the suckers.

The material from ratoon fruits and tops was most variable; fruits were of various sizes and conditions, most of them stunted and growing on plants that varied considerably in succulence. The plots of this series varied most in amount of collapse. Examination of all these plots revealed no relationship between green spotting and amount of collapse. Mealy bugs from wilting young plants produced green spotting in only 1 plot out of 9 on January 4, while plots infested with mealy bugs from old, green-spotted ratoon-field plants showed green spotting to be general.

Preliminary tests of the effect of a reversed host sequence are described in Experiment V.

Experiment V. Preliminary tests of the effect of host sequence on mealy-bug toxicity

Since experiments on toxicity of mealy bugs from various sources had indicated a relationship between toxicity and host plant or host-plant condition, the following experiment was performed in the laboratory.

A mealy-bug colony from wilting suckers was divided into 3 parts. One aliquot was placed on Panicum grass and, after feeding thereon for 9 days, the mealy bugs were transferred to healthy pineapple plants, 50 to each of 5 plants. A second aliquot was transferred to the leaves of another pineapple plant in advanced wilt. These leaves were separated from the plant and kept in tubes, as shown in figure 7, C. After feeding on these wilted and drying leaves for 19 days the mealy bugs were transferred, 50 to each of 4 healthy pineapple plants. The third aliquot was transferred directly to healthy plants, 50 mealy bugs to each of 5 plants. The results of this experiment are shown in table 9.

A colony of mealy bugs from red-top Natal grass was divided. Some were placed directly on healthy pineapple plants, 50 to each of 5 plants. Another aliquot was fed on leaves from the same badly wilted and green-spotted pineapple plant used in the last-named experiment. After 11 days on these leaves, the bugs were transferred in the same manner to 3 healthy pineapple plants. Neither transfer resulted in either wilting or green spotting.

TABLE 9.—*Effect of host-plant sequence in modifying toxicity of mealy bugs*

Host sequence	Number of plants used	Wilt ensuing
Wilting suckers direct to healthy plants	5	4[a]
From wilting suckers; to Panicum grass for 9 days; thence to healthy plants	5	0[a]
From wilting suckers to leaves of a plant in advanced stages of wilt of 19 days; thence to healthy plants	4	1 partial wilt[a] Edges of leaves on remaining plants reflexed inward

[a] All these plants were green-spotted.

In view of the small number of plants used, the evidence of these experiments can be considered only as preliminary. It is, however, rather clearly indicative. The wilt obtained was typical and pronounced, so the complete absence of wilt in the plants fed on by the mealy bugs from wilting suckers *via* Panicum grass indicates the profound effect of changes in the insect's nutrition on the toxicity of its secretions. The fact that mealy bugs will forsake a wilting plant is evidence of the unsuitability of such plants as hosts; so, the results obtained by forcing the insect to feed on a wilted leaf for a considerable time are similarly indicative of a modification of the insect's secretion.

Comparison of Green-spotting and Nongreen-spotting Mealy Bugs in Relative Capacity to Produce Wilt. Field observation had shown that, while green spotting was generally to be found in wilted areas, it was not necessarily always associated with wilt, even under field conditions. As has been mentioned, some of the mealy bugs used in the above experiment came from wilted plants showing no green spots. Other areas on the island of Oahu revealed the same to be true, even though of rare occurrence. On the island of Kauai, however, there are areas that have been under intermittent observation for the last 2 years and have shown no green spotting, although typical wilt associated with high mealy-bug populations is common. A laboratory experiment was, therefore, set up, using mealy bugs from wilting plants from 1 of these areas on Kauai in comparison with typical green-spotting mealy bugs from wilting plants on Oahu.

The conclusion was definitely established from this experiment that typical wilt of pineapples follows feeding of *Pseudococcus brevipes* irrespective of green spotting. The development of the wilt obtained in both series indicates clearly the limited extent of the injury, since, with the great

reduction in size of colonies, most of the plants had normal center leaves after 5 months.

The results indicate, however, that in the presence of green spotting, wilting is somewhat more severe; though such evidence as there is on this point may be explained as due to the much more rapid establishment of the green-spotting colonies. The green-spotting colonies established more rapidly and have maintained themselves with much greater vigor than the non-green-spotting colonies.

In May, 1932, these latter colonies were reduced in every case to a few scattered individuals, while the green-spotting colonies were vigorous and, in some cases, very large. Evidence of the existence of biological strains of mealy bugs is considered in more detail in another paper (5). The matter is mentioned here, since such strains, or perhaps even physiological states, of a colony, may be of importance in determining the toxicity of the colony to pineapple plants.

Recovery of Wilted Plants. This is a phenomenon that has been consistently encountered during the course of this study. Notes are included in tables 3 to 7 on the recovery of plants under several conditions of infestation and subsequent collapse. In all the infestations of 50 mealy bugs to the plant made in Experiment II on February 3, 1931, recovery followed the apparently complete collapse of the plant. This manifested itself in the production of new leaves in the center, normal in color, but very narrow and somewhat brittle, giving the effect of spindly growth noted in the tables. This new growth, while of no significance commercially, is of great interest in that it demonstrates that the meristematic tissue was not injured.

The plots infested April 27, 1931, with 50 mealy bugs showed this recovery in a much greater degree. The most clean-cut example is shown in figures 4, A, and 11. This series of photographs of the same plot illustrates the gradual recovery from an apparently complete collapse. A laboratory experiment was performed on 5 of the plants from this plot, which accounts for the blanks seen in figure 11. These plants were pulled and the dead roots and old butts trimmed off. The plants were then set in culture solutions. All promptly produced apparently normal roots. This series is shown in figures 4, B, and 7, B.

The recovered plants in this plot, at the time of photographing, figure 11, B, harbored colonies of mealy bugs that obviously totaled more than the 50 of the original infestation. It should be recalled that the colonies developing on these plants practically disappeared with the collapse of the plants, so that the colonies found on the recovered plants represented a gradual building up from the few remaining on the plants. The fact that the plant could recover to the extent shown is a remarkable instance and is strongly suggestive of an antitoxic or accommodatory reaction on the part



FIG. 11. A. Same plot as in figure 4, A. Photographed Nov. 20, 1931. B. The same plot photographed Feb. 9, 1932.

of the plant. On April 11, 1932, 1 of the plants in this plot was beginning to wilt for the second time; the balance showed no wilt symptoms.

Recovery on the plots of Experiment II was of interest in indicating the relationship between age of the plant at the time of collapse, and sub-

sequent recovery. Observations, made April 11, showed that recovery had taken place on the plants of the first infested plot to the extent of permitting the development of small but otherwise normal fruits on 3 plants and apparently normal but small inflorescences on 13. The plot infested 3½ months later showed 6 plants wilting on February 16, which, on April 11,

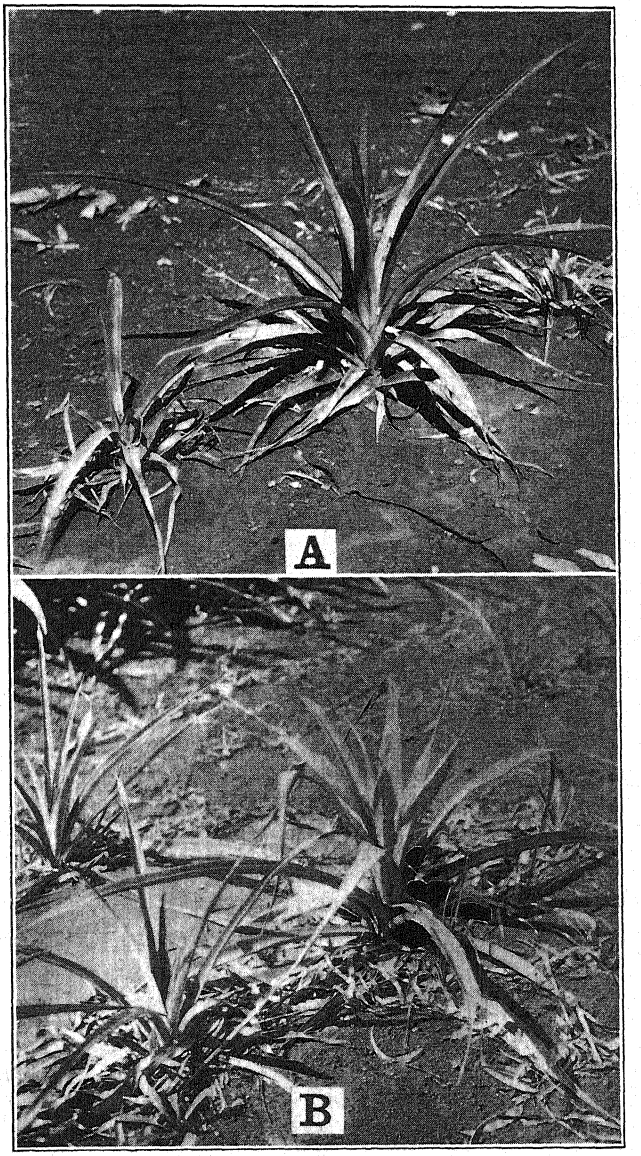


FIG. 12. A. Plant showing resistance to quick wilt. July 4, 1931. B. Collapse of plant in A, from slow wilt Nov. 20, 1931. Note recovery of contiguous plants.

were recorded as bearing normal fruits. All the other cases of wilt recorded February 16 in this plot showed either no further growth or an attempt at recovery, which culminated in a small inflorescence, at which point the plant ceased growing and the inflorescence dried up.

When pineapple plants have collapsed from slow wilt, recovery apparently does not occur. This was particularly evident in plot 1 of table 3. The other plots of this series showed some spindly recovery, but these were the plants that had been the first to collapse. Plot 5 of table 4 had 1 plant that did not develop quick wilt, while all the surrounding plants did. On July 4, 1931, this one plant was outstanding, as shown in figure 12, A. Following this, a very large colony developed, although the plant attained large size. In the meantime the collapsed plants in the same plot began to show spindly recovery. On November 20, 1931, as shown in figure 12, B, the large plant had collapsed from slow wilt.

Three other expressions of recovery should be mentioned. If the mealybug colony is short-lived or disappears entirely after collapse of the plant, the center whorls of leaves are normal in color, except for reddened or chlorotic areas at the tips. As the plant grows the new leaves are normal and the originally affected whorls show tip reddening only. This type is frequently encountered within fields where colonies have started on infested planting material and have died shortly afterwards. Another type follows recovery from quick wilt in 8- to 10-month-old plants (Figs. 1 and 5, A, and B). It is characterized by brittle, narrow, new, central leaves with a reddish brown cast. This condition is more likely to be confused with plant failures due to other causes than any of the previous types. The third type is characterized by the occurrence of wilt in early fruiting; the production of normal suckers in these cases is a commonly encountered phenomenon. It should be borne in mind that none of these recovery types are of any significance commercially.

Evidence for the Plant's Accommodation to Mealy-bug Feeding. As has been shown in Experiment II, tables 3 to 6, a suddenly applied colony of 50 mealy bugs produced wilt quickly in plants from 6 to 11 months old. On the other hand, some plants infested with 1 gravid female grew to a large size, even though colonies comprising over 1,500 individual mealy bugs developed before the plant finally succumbed to wilt. Some plants, similarly infested, but that had by November 20, 1931, developed colonies of approximately 500, produced a large percentage of normal-size fruits. Since the mealy bugs were all taken from the same source, these differences cannot be explained on a basis of difference in individual mealy-bug toxicity when the insects were first placed on the plants.

To test this, some of the mealy bugs from one of the large colonies were taken on December 17, 1931, from a still healthy plant in plot 1 and each

of the plants growing in soil and under cages was infested with 50 of these mealy bugs. Another lot of plants was similarly infested with mealy bugs from the most toxic source known, *i.e.*, wilting first-ratoon suckers. There were 9 plants in each lot. Observations made up to April 19, 4 months after infestation, revealed essentially the same results from each. Complete as well as partial collapse occurred in both series in the same lapse of time. It was evident from this experiment that both lots of mealy bugs were equally toxic.

Evidence of the ability of the plant to withstand a large population of mealy bugs for a long time, if the colony started from a very small initial infestation, can be commonly seen in any of the pineapple areas in Hawaii. The fact is of great importance, for, while quick wilt is extremely difficult to stop, once mealy-bug colonies are established on pineapple plants, the holding down of mealy-bug populations to a very low level is practical and is being accomplished. Further experiments on the subject of the accommodation of the plant to mealy-bug attack are now under way.

The Possible Nature of the Injury. Mealy-bug wilt of pineapple appears to present a type of insect-induced plant disease for which it is difficult to find a parallel case, although there are points of similarity with hopperburn of potatoes (7) and sugar-cane froghopper blight in Trinidad (18, 19). There is no positive evidence of either a virus or a "transmissible" disease, transmissible in the sense of being transferred by insect "pick up" from 1 plant to another. On the other hand, there is definitely negative evidence to the contrary.

Vegetative planting material from areas that have collapsed shortly after removal of the slips has been used for years in the ordinary plantation practice. This planting material has almost invariably been infested, at time of planting, with mealy bugs from the same colonies as those that produced wilt in the parent plants. The relationship between size of initial infestation and later behavior of the plant indicates clearly that a time-quantity relationship holds. The remarkable recovery of the plant is further evidence that we are not dealing with either a virus or a transmissible disease, in the limited sense. It would seem that the injury is more likely to be a toxic secretion that is diffusible but incapable of reproducing in the plant. The accommodation of the plant to large but gradually developed colonies is inexplicable if a living systematically distributed entity is postulated as causal.

The speed and completeness of the collapse in the case of plants that have been subject to a sudden and heavy attack indicate a systemic condition, but the recovery of these plants as shown in figures 4, A, and 7, B, shows that, even in those cases, the toxic principle did not kill either the

root primordia or the meristematic tissue at the base of the new leaves. The cases of terminal wilt appear to be examples of restricted diffusion of the toxin.

Whether quick and slow wilts are expressions of the same causality cannot be definitely determined now; the latter case may be due to a combination of a weakening of the plant's accommodation or the gradual elimination of the functional leaf tissue or both. The latter, as a single factor, is unlikely in view of the fact that the plant can withstand the removal of a large proportion of its leaf tissue without untoward effects.

Data on the influence of source of mealy bugs and subsequent plant collapse indicate differences in degree rather than kind and suggest what seems to the writer to be a reasonable hypothesis of great significance if true. That is to say, the toxicity of the insect's secretion varies with the nutritional conditions under which the insect is living. This toxicity decreases after reaching a peak attained shortly after plant-crop harvest, so that mealy bugs from old ratoons are actually less toxic than those that have developed on the extremely succulent plant-crop tissue. No conclusion as to the nature of the injury is yet possible. The question has merely been opened up for further work.

Mealy Bugs in Relation to Other Inimical Factors. Although *Pseudococcus brevipes* has been shown by laboratory experiments to be a primary and single factor in causing wilt, with symptoms similar to those found under field conditions, it still remains true that, in the latter case, mealy bugs are operating in the presence of a biological complex that may condition their effect on the plants. Since time-quantity relationships hold for mealy-bug toxicity, it follows that the presence of other inimical factors might reduce both the amount of mealy-bug infestation necessary and the time over which it operates to produce wilt. On the other hand, mealy-bug-wilt symptoms might mask the effects of other factors that might, themselves, be important contributors.

Evidence is accumulating, however, from a large series of control experiments, as well as from commercial fields that have been consistently treated for mealy-bug control, that mealy-bug wilt is the major factor in failure of pineapples in Hawaii and that other factors only operate in extremely localized areas and sometimes then only under abnormal weather conditions.

SUMMARY AND CONCLUSION

The pineapple mealy bug, *Pseudococcus brevipes,* has been shown by laboratory and field experiments to be primarily responsible for a widespread collapse of pineapple plants. Since symptoms are characteristically of the wilt type, the term "mealy-bug wilt" is a suitable designation.

Green spotting of pineapple leaves is a localized effect at the insect's feeding point and not a typical wilt symptom. Wilt is more pronounced, however, where green spotting is present. Ants are important contributors to the vigor of a mealy-bug colony and the incidence of wilt is low where these species, notably *Pheidole megacephala* and *Solenopsis geminata* var. *rufa*, are absent.

The disease assumes many forms, dependent upon the size, time of onset of the initial mealy-bug population, the vigor and succulence of the plant, and the fact that recovery in various degrees is commonly encountered.

Quick wilt is the type that follows a sudden attack by a number of mealy bugs. Approximately 2 months are required for development of typical symptoms. The establishment of the mealy-bug colony is not necessary to produce quick wilt, a short period, only, of feeding by the insects being sufficient. Under these circumstances there is a rapid diffusion of the toxic principle. This diffusion may be localized to the leaf area actually fed upon or it may affect 2 or 3 leaves or the entire plant. It does not, however, kill the meristematic tissue either of root primordia or younger leaves. Susceptibility to quick wilt decreases under conditions of reduced vigor and succulence and age of the plant.

Recovery from quick wilt is commonly encountered. It is usually of no commercial significance but sometimes results in the production of small but otherwise normal fruits.

Evidence of antitoxic reactions on the part of the plant is available, for when a colony develops from a single gravid female, no quick wilt occurs, even though enormous numbers of mealy bugs are feeding on the plant. If such a colony continues over a period of many months, slow wilt may occur, from which there is no recovery. The size of the colony and the length of time it operates determine whether slow wilt will occur.

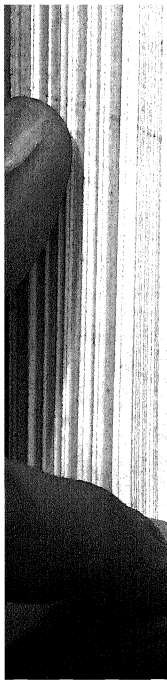
There is definite evidence that the mealy bug's toxicity varies with the kind and condition of the insect's host plant. This is believed due to changes in the insect's secretions induced by the varied nutritional conditions afforded by the host plants.

Mealy-bug wilt appears to be an insect-transmitted disease for which no closely parallel case is known. The evidence points to a nonliving toxic insect secretion of variable diffusibility as causal.

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THE SPOTTING OF PINEAPPLE LEAVES CAUSED BY PSEUDOCOCCUS BREVIPES, THE PINEAPPLE MEALY BUG[1]

WALTER CARTER

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INTRODUCTION

The green spotting of pineapple plants caused by the feeding of pineapple mealy bugs, *Pseudococcus brevipes* (Ckl.), is conspicuous in many pineapple areas in Hawaii. It was considered a characteristic symptom of pineapple wilt by Illingworth (3). Experimental evidence obtained by the writer and recorded in another manuscript (1) led to the conclusion that "typical wilt of pineapple follows feeding of *P. brevipes,* irrespective of green spotting," even though wilt appears to be more severe in areas where green spotting is the rule. This conclusion is also supported by much field observation. For the purpose of this study, therefore, the phenomenon of green spotting has been considered as something separate and distinct from pineapple wilt. For purposes of comparison, the injury following the feeding of nongreen-spotting mealy bugs was studied also.

TYPES OF SPOTTING RESULTING FROM MEALY-BUG FEEDING

A. *Green-spotting Bugs.* Like other members of the genus, *Pseudococcus brevipes,* though capable of active locomotion, normally feeds at one place on the leaf for some considerable time. If a leaf is pulled out of the center of an infested pineapple plant, the mealy bugs can be seen *en masse* near the white tissue at the proximal end of the leaf. This relative position is maintained unless the colony becomes very large, in which case it extends up the leaf for some distance.

In this white tissue, and especially at the poorly defined junction of green and white tissue, faint yellow-green homogeneous spots appear. As the leaf grows out, these spots retain their relatively darker color than the surrounding tissue. By the time the leaf area in which they are has become normally green these spots are clearly defined, but they vary a good deal both in initial appearance and development. Early laboratory tests indicated that green spotting was localized around the insect's feeding point. Tests to determine this were made by caging mealy bugs on small areas of pineapple leaves, as shown in figure 1, A. Spotting consistently occurred, but only on those areas where the insects had fed.

[1] Published with the approval of the Director as Technical Paper No. 49 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

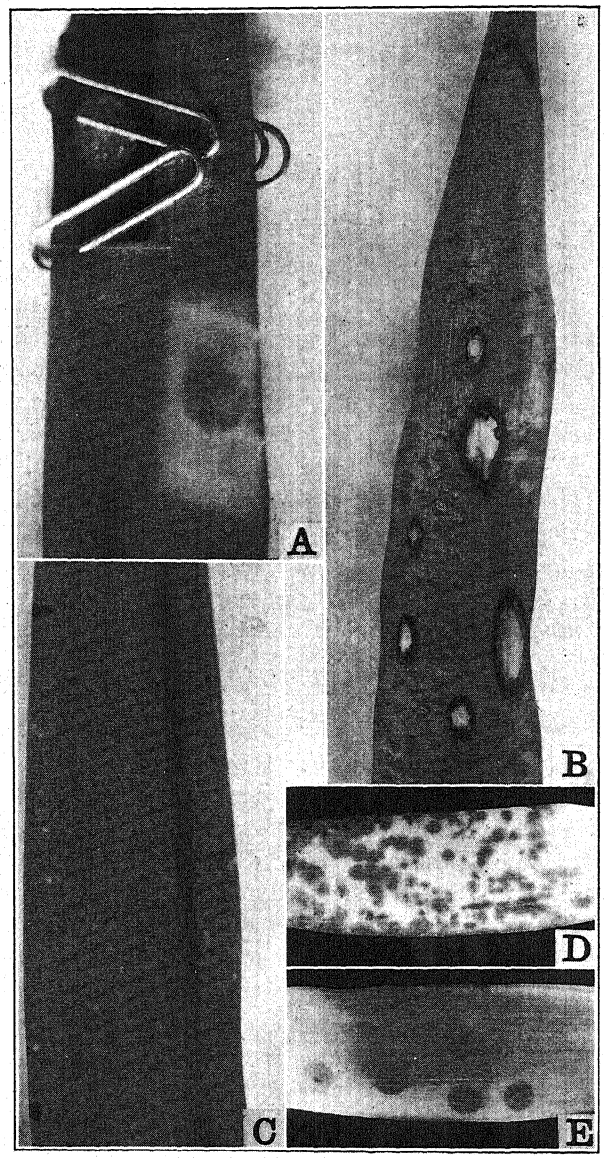


FIG. 1. Portions of pineapple leaves, A and D being from seedling leaves. A. Area with a felt-celluloid cage for confining mealy bugs and one showing green spots confined to portion that bugs fed upon. B. Homogeneous spots on margins, welt-like spots in median area and areas of secondary necrosis. C. Disappearance of outer green zones from leaf marked when spots were normally zonate. D. Fixation of color in mass of green spots and extremely chlorotic areas between them. E. Large green spots with dark centers. At left note the spot in the chlorotic tissue at proximal end of leaf.

The principal forms taken by these spots are listed below and illustrated in figures 1, B–E, and 2, D.

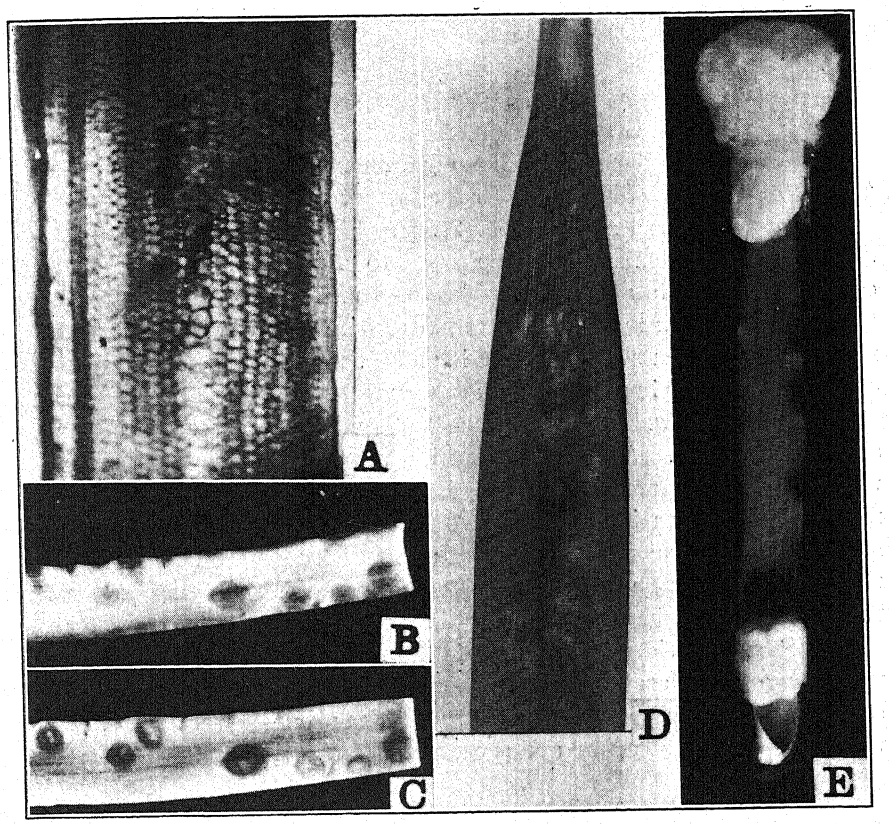


FIG. 2. A. Section through chlorotic spot, showing thickened cell walls and densely staining areas filled with homogeneous mucilage-like substance. B. Green spots with dark centers on seedling leaf. C. Same leaf as in B, after clearing in 95 per cent alcohol and staining in iodine-potassium-iodide solution. Note dense staining of green spots and their light unstained centers. D. Grouping of chlorotic spots in areas of irregular margin. E. Tubed leaf of pineapple seedling used in laboratory experiments.

1. A faint, usually circular, homogeneous spot of yellow-green in the white tissue of the leaf. Frequently 2 or more in a small group. *2.* Homogeneous, usually circular, darker green area in the young, green tissue; sometimes slightly depressed. *3.* As in 2, but with a concentric zone of lighter green around the darker center. This lighter zone is still darker green than the normal tissue. Frequently the outer zones of 2 or more of these spots will merge. The zonate character of a spot may appear with the first sign of spotting, or much later. *4.* Green welts. The green spot

becomes slightly raised in somewhat older tissue and is particularly conspicuous in chlorotic leaves that have shrivelled. Occasionally these welts develop a small browned center of secondary necrosis. 5. The darker center of the zonate spot becomes chlorotic and the outer zone either disappears entirely or nearly so. When the leaf shrivels and becomes chlorotic these spots show as faint green welts. Spots may be chlorotic-centered from the beginning, but this is rare.

The spots vary from approximately 1 mm. to 10 mm., the average being from 3 to 5 mm. in diameter. In the largest one encountered, the darker center was ovoid and measured 4×2 mm. Size of spots is sometimes associated with a particular mealy bug or group of mealy bugs. One small colony produced spots on a seedling leaf 6–8 mm. across. This size of spot was maintained through several transfers, finally, however, appearing normal in size. When a large colony is present the leaf is sometimes literally covered with spots in all stages of development.

B. *Feeding Marks of Nongreen-spotting Bugs.* These are of a much simpler character. A nongreen-spotting mealy bug leaves either no definite mark or a chlorotic spot, sometimes circular but usually of irregular margin and very variable in size. A large colony will sometimes cover the leaf with such areas so that it appears almost translucent. In these cases there is usually some rugosity due to the irregular shrinking of the leaf. The chlorotic spots usually develop a brown necrotic center.

While an individual green spot is the result of the feeding of a single mealy bug, the chlorotic area is more usually the result of a mass of mealy bugs feeding in one very localized area for some time—several days, or even weeks, in the case of young and succulent plants.

PRELIMINARY STUDIES OF THE ANATOMY OF THE SPOTS

In view of the variety of forms of green spotting and the complexity of the problem as revealed by transmission studies, anatomical studies have been limited to the grosser features with a view to comparing the chlorotic with the green-spotted area as a means of establishing the specific identity of the 2 types.

Mealy-bug Feeding Tracks. The feeding tracks left by Homopterous insects in plant tissue have been studied in detail by other workers (2, 4, 6). The tracks of *Pseudococcus brevipes* are readily found in both types of spotting. They are remarkably uniform in width and surface. They penetrate intracellularly to the region of the fibrovascular bundles where they may branch, each branch leading to a separate bundle. Rarely a track may pass through the entire width of the leaf, curving back without interruption to a point near the fibrovascular bundles where branching may occur.

No differences in general morphology have been observable between feeding tracks in green and chlorotic-spotted areas. In view of the conclusion reached that the 2 types are the result of differences in secretion, a comparative microchemical study of feeding tracks should prove fruitful.

The Chlorotic Area. Sections through these areas show degenerated chloroplasts (Figs. 2, A, and 3). When stained with iodine-potassium

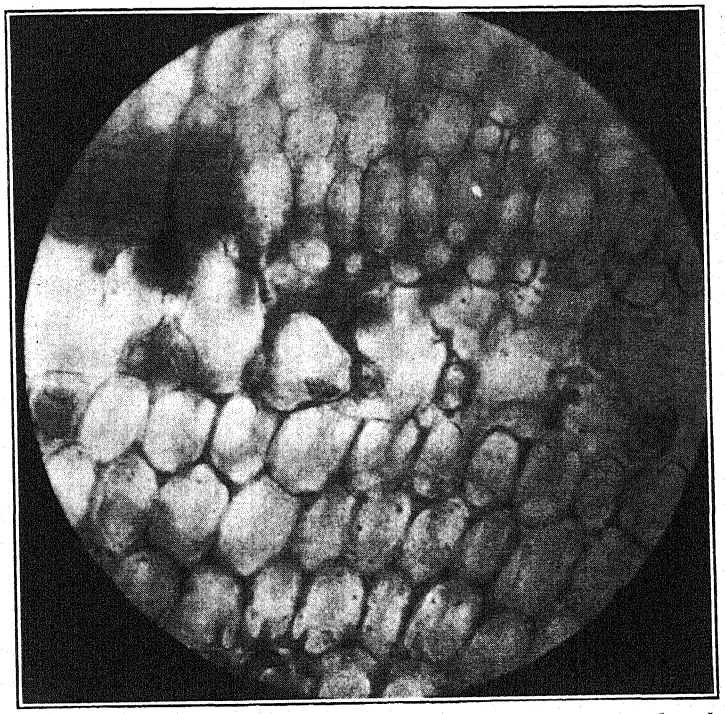


FIG. 3. Enlargement of portion of figure 2, A, showing degenerated chloroplasts, cell-wall thickening, and densely staining cell contents.

iodide it is seen that the area is free of starch. Stained with dilute safranin, a thickening of the cell walls can be clearly seen. This varies in extent, being more pronounced in the central portion of the area and especially so when the center of the spot appears browned with secondary necrosis. This central area, in section, showed heavier vein thickening from the epidermis to the fibrovascular bundles. The finding of old feeding tracks in this area suggests the probability of its developing around the feeding track, the reduced thickening towards the periphery being due to a diffusion of the secretion in that direction. Within this area of pronounced vein thickening, many cells are full of a homogeneous nongranular substance that takes a slightly less dense stain than the thickened cell walls. Differences be-

tween chlorotic areas on the same leaf and on different plants appear as slight variations in the extent of vein thickening.

Green Spots. These present an entirely different morphological picture. In the earliest stages, when the green spot shows as a faint yellow-green area in the white tissue, sections show the area to be a zone of hyperactivity. The chloroplasts are clearly formed within the area that is surrounded by normal white tissue in which no chloroplasts are to be found. Stained with iodine-potassium iodide, after clearing in 95 per cent alcohol, the chloroplasts in the area of the spot become deeply stained, so that the contrast between it and the surrounding tissue is heightened.

Green spots within normally green leaf areas show this same deeper staining with iodine-potassium iodide with a diffused muddy-brown color throughout the area of the spot, as well as the deep-stained chloroplasts. When stained with 1 per cent acid fuchsin without clearing the leaf, a diffused pink fills the area. When stained after clearing, it is pale lavender, with scattered cells throughout the normal leaf tissue filled with diffuse pink. There is a curious difference in the case of spots having a deeper green central zone. When such a leaf is cleared and stained with iodine-potassium iodide, the stained area is exactly coincident with the area of the spot except for the central zone, which shows no evidence of starch (Fig. 2, B and C). This central area is that through which the insect's mouth parts actually punctured the leaf.

No evidence has been obtained to indicate thickening of cell walls or other injuries, as seen typically in the chlorotic area, in any of the forms of green spot. The typical condition in these is enlargement and increase in number of chloroplasts. When tissue is cut from a green spot and macerated on a slide, flat plate-like polygonal bodies, with irregularly thickened edges, are to be found. The bodies stain deeply with iodine-potassium iodide. Similar treatments of normal tissue from the vicinity of the spot show these to be absent.

The green welt, isolated in a completely chlorotic area at the drying tip of a leaf, presents a curious picture. In such a spot the cells appear still to be living, even though the cells of the surrounding tissue are dead and shrunken.

The Occurrence of the Two Types in the Field. Field observations on the islands of Oahu and Kauai suggested that green spotting was not necessarily a concomitant of mealy-bug feeding, even where pineapple wilt was present. In a considerable portion of the pineapple area on Kauai, no green spots could be found, even in badly wilting plants. In other places green spotting is rare, only very small pinhead-size spots being found and they only occasionally on any one plant. In other areas, notably in the drier lands, green spotting is the rule. Plants carrying nongreen spotting

colonies are occasionally found on all the islands of the group, but green spotting in greater or less degree is the rule on all the islands but Kauai. Observations on an experimental plot on Oahu in the spring of 1931 revealed large colonies present but no green spotting along a field edge of approximately ½ mile, except in 1 small plot. Typical wilt was associated with the chlorotic type of feeding spot. Occasionally, 2 plants growing in extremely close proximity will show green spots on the one and not on the other. Green spotting will sometimes appear on the plants for the first time on the crowns of the fruit.

Mealy bugs from hosts other than pineapple were tested for green spotting. Two of these host plants, Panicum grass, *Panicum barbinode,* and red-top Natal grass, *Tricholaena rosea* Nees, are important sources of mealy-bug infestation since they are frequently found on the edges of fields, and colonies from these 2 species have been maintained on pineapple seedlings over a considerable period. No positive evidence of green spotting by colonies from either grass has yet been obtained. Two species of sisal are typically green-spotted by *Pseudococcus brevipes,* and transfers of the insects to pineapple resulted in the prompt green spotting of the leaves. *Pseudococcus brevipes* from the roots of purslane, *Portulaca oleraceus* Linn., and Rhodes grass, *Chloris gayana* Kunth, produced green spots when transferred to pineapple-seedling leaves. The negative evidence obtained from a number of other hosts must be considered as only tentative in view of the later finding that the capacity to produce green spots may be limited to certain individuals in a colony.

As these differences in the field became evident, samples of the mealy bugs found both on pineapples and other hosts were brought to the laboratory for study.

Technique of Laboratory Studies. Seedling-pineapple plants were used, grown in soil, and enclosed in glass jars with cloth tops. Mealy bugs from the field were transferred to these and the development of spotting noted. In order to study the comparative effect of individuals of the same colony, some of these seedlings were dissected and the largest leaves placed in a small quantity of culture solution in a sterilized test-tube (Fig. 2, E). This type of tube proved most useful since green spots developed readily and reproduction of the mealy bugs took place. Sterilizing of the prepared tube prior to enclosing the leaf very much reduced secondary infections, but the type of culture solution was immaterial. With the rapid corking of the proximal end of the leaf it is doubtful whether the solution does more than prevent desiccation.

The seedlings were grown in the glasshouse while the tube colonies were held under various laboratory conditions of light and temperature. Green spots developed on leaves placed in total darkness at the time of infestation

and also on leaves held in total darkness for 1 to 2 weeks prior to adding the insects. Most of the experimental colonies grown in tubes were placed upright in cans under conditions permitting diffused light only to reach the leaves.

EXPERIMENTAL WORK

The first question was whether green spotting was merely a reaction of individual plants. This was tested in 2 ways: first, by feeding green-spotting mealy bugs on the leaves from which nongreen-spotting mealy bugs were removed; second, when mealy bugs failed to produce green spots on seedlings, the seedlings were dissected and the individual leaves tubed. Some of these leaves were then reinfested with the same bugs as a check on the changed conditions under which the leaf was then growing, while green-spotting bugs were transferred to the balance of the separated seedling leaves. These experiments resulted consistently in evidence that the difference between green and nongreen spotting was due to differences between mealy bugs.

Establishment of a Consistently Nongreen-spotting Colony of Pseudococcus brevipes. A mealy-bug colony taken from a first-ratoon sucker growing in a Haleakala Ranch field on the Island of Maui had produced no green spots on the field plant. The colony was transferred to seedlings and later to a long series of tube colonies. The pedigree of this colony is illustrated in figure 4. It definitely establishes the fact that *Pseudococcus*

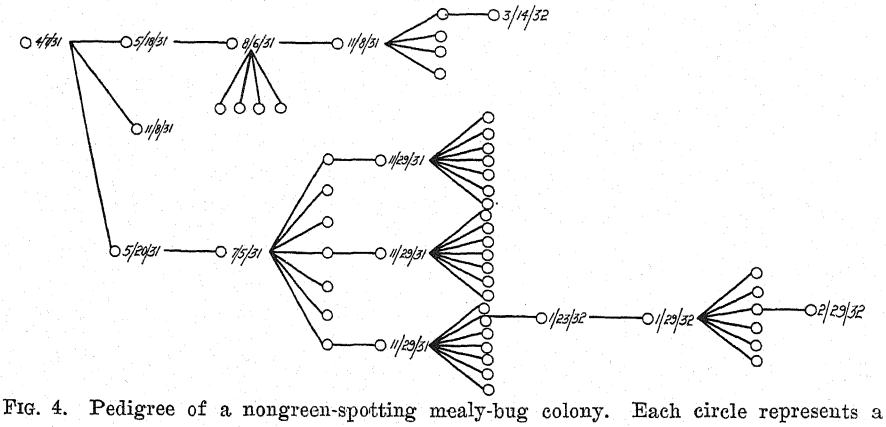


FIG. 4. Pedigree of a nongreen-spotting mealy-bug colony. Each circle represents a separate colony grown on seedlings and seedling leaves.

brevipes colonies may exist for a long time and on a long succession of pineapple seedlings without any green spots resulting.

Another pedigree of a nongreen-spotting colony from the field edge on Oahu mentioned above presents some interesting possibilities. It will be seen (Fig. 5, B) that occasional cases of green spotting appeared. This

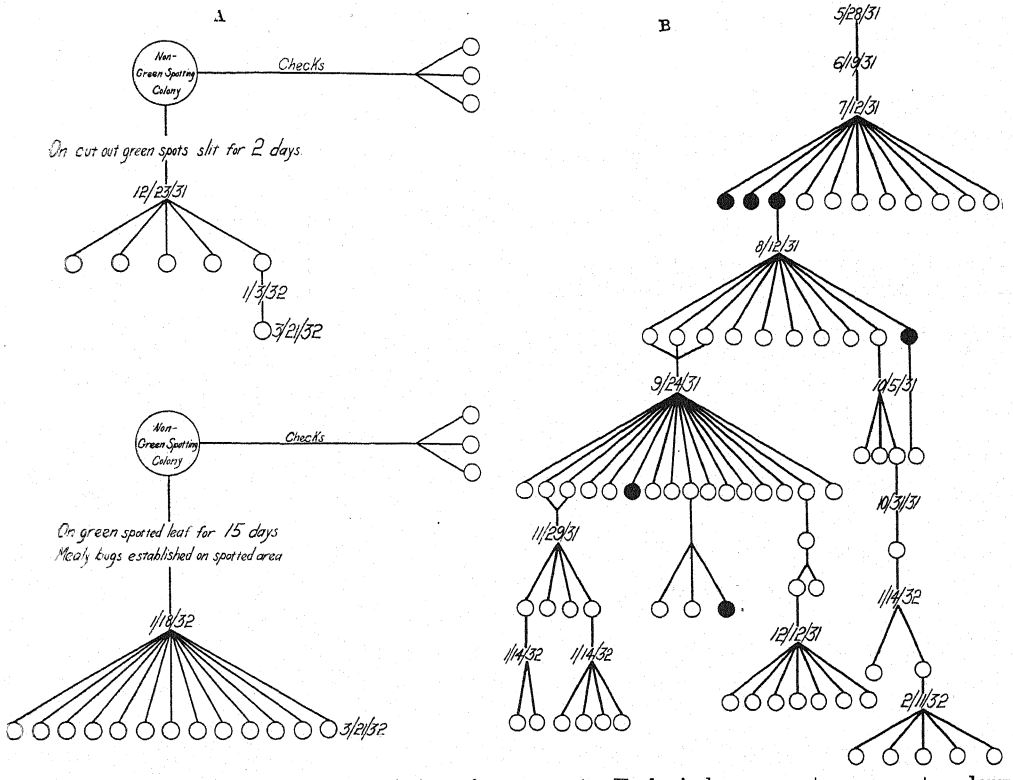


FIG. 5. A. Experiments on transmission of green spot. Each circle represents a separate colony on seedling leaves, none of which showed any green spotting. B. Pedigree of a mealy-bug colony, showing occasional occurrence of green spotting. Green-spotted plants are shown as solid circles.

indicates the possibility of colonies being inherently capable of producing green spots but having this capacity in a recessive condition.

Continuity of Green Spotting by Colonies and Individual Mealy Bugs. Colonies have been maintained on single plants for periods of up to 1 year with a continued successive appearance of green spots. Occasional examples have occurred where green spots have failed to appear on the new growth, but, when the insects were transferred to new plants, green spots appeared on them some time later. Colonies removed from wilted pineapple plants that were badly green-spotted on all but the newest tissues gave variable results. Some aliquots of the original colonies produced green spots on young seedlings and others not. It should be noted in all these cases that the colony rather than the individual mealy bug has been the unit.

Individual mealy bugs have been fed on a succession of separated pineapple leaves, being transferred after feeding for various lengths of time. This series is shown in table 1.

TABLE 1.—*Sequence and number of green spots obtained from individual mealy bugs*

Series	Days of feeding							
	1	3	6	3	9	6	3	8
1	0	0	0	1	2	0	0	
2	1	0		0	0	Progeny produced spots	Progeny produced spots	
3	3	1	3	1	2	0		
4	1	1	1	5	7	4	1	2
5	0	1	1	0				
6	1	1	1	1	1	2	1	Progeny produced spots
7	0	0	0	2				
8	1	1	1	0	0	2	0	
9	1	1	1	2	1	2	0	0
10	1	1	0	1	0	Progeny produced spots		Progeny produced spots
11	1	0	2	0	0	0	0	0
12	0	0	0	0	0	0	0	0

In this table the number of green spots developing on each leaf is shown. It will be observed that there was a good deal of variation between individual insects, 1 individual not producing a single spot during the whole period. This is of interest, since all these mealy bugs came from a very profusely spotted leaf. They were, however, variable in size and age and several became mature during the course of the experiment. It is apparent in following the course of mealy-bug development that the gravid female feeds but little prior to the emergence of her young, so that negative results towards the end of the insect's development may be primarily due to reduced feeding.

The green spotting by the progenies of 3 individuals should be noted, since it occurred on the same leaves on which parent insects failed to produce spots.

Development of the Green Spots. Since green spots are determined by comparison with the surrounding tissue, the initial density of the chlorophyll and the rate at which the leaf becomes chlorotic are factors that affect the time elapsing between feeding of the insect and the appearance of the spot. When data of table 1 were obtained, records of the time elapsing between the first application of the insects and the development of the spot showed this to vary from 3 to 55 days. Occasionally, the spot develops while the insect is still feeding, but usually the spot appears after removal

of the insect. It seems true that a green-spotting bug moves its feeding point much more frequently than one unable to produce spots.

TRANSMISSION EXPERIMENTS

When it became evident that capacity to make green spots was limited to certain mealy bugs, experiments were performed on the transmissibility of green spotting.

Mechanical inoculation with macerated plant tissue, containing spots and with mealy bugs that had produced spots, was performed on growing seedlings and on separated seedling leaves. A large series gave negative results. In one case a large circular green spot resulted from hypodermic injection of macerated green-spotted tissue. This was interpreted as a wound reaction with which the metal of the needle might have been concerned, inasmuch as deep green areas frequently resulted from cracking of the leaf as it was being inserted into the tube and from the insertion of glass capillaries, which resulted in small necrotic areas with margins greener than surrounding tissue.

Experiments on transmission of green spot by first feeding nongreen-spotting individuals on green-spotted leaves gave negative results.

In the first of these experiments green spots were cut out of a leaf and partly slit with a razor blade. This was done to encourage the localization of the mealy bugs' feeding to the deeply spotted portion, since *Pseudococcus brevipes* has a tendency to establish in cracks and crevices. This localization occurred and the insects fed for 2 days before being transferred to seedling leaves (Fig. 5, A).

A second experiment using nongreen-spotting mealy bugs was then set up. Gravid females were placed on a heavily spotted leaf. The larvae from these females settled down on green spots and fed thereon for 15 days. They were then transferred to seedling leaves as shown in figure 5, A. Although the results of these transfers were all negative, they were of considerable significance in view of the success obtained in localizing the feeding of the insects to the actual green-spotted tissue.

THE HEREDITARY NATURE OF THE CAPACITY TO FORM GREEN SPOTS

This species of mealy bug, being viviparous, gives birth to living young. Gravid females, when placed in a glass container, such as a Petri dish, will give birth to numbers of these larvae, which can, therefore, be obtained without any previous feeding on plant tissue. Particular care was exercised that no larvae were adhering to the parent insects when the latter were transferred to the Petri dish. When unfed larvae from green-spotting mothers were transferred to clean seedling leaves extensive green spotting occurred. This experiment was repeated twice with the same results. Table 2 gives the data on one of these experiments.

TABLE 2.—*Production of green spots by larvae that had not previously fed*

Larvae transferred to seedling leaves 11/23/31	Observation dates		
	12/20/31	1/3/32	1/14/32
Seedling A.1[a]	−	+	
.2	+		
.3	+		
.4	−	−	+
.5	−	+	
.6	−	−	+
.7	−	+	
B.1	+		
.2	−	−	+
.3	−	+	
.4	+		
.5	+		
C.1	+		
.2	−	+	
.3	+		
D.2	−	+	
.4	+		
.5	+		
Larvae transferred to seedling leaves 11/26/31			
E.1	+		
.2	+		
.3	+		
.4	+		
B.6	+		

[a] Each decimal number represents a single separated leaf. The leaves of 5 seedlings were thus used in this experiment.

The dates when the larvae were removed are given, as well as the dates on which green spotting occurred. Removal of larvae from the glass dish on the 2 dates shown was for the purpose of testing the persistence of the green-spotting capacity through the entire brood. The results of the last removal show this to be the case.

EVIDENCE FOR EXISTENCE OF GREEN-SPOTTING STRAINS

In the colony pedigrees presented in figures 4 and 5, B, it is seen that colonies were maintained for a year without the appearance of any green

spots; in others, with occasional incidence of green spotting. Other colonies have consistently green-spotted the leaves, with some evidence of fluctuation in numbers of spots produced in any one period.

Observations in the field during January, 1931, revealed what was believed to be a high percentage of fungous parasitism of mealy bugs. Some large gravid females were black from the filling of the body with fungus spores. These were comparatively rare, but numerous other individuals could be differentiated from the rest of the colony by a distinct grayness as compared with the normal rose pink of the other individuals. These gray individuals were presumed to be in early stages of the disease, so samples were isolated on separated leaves in tubes. A similar set was made up, using typically pink individuals from the same large field colony. Six weeks later examination of both sets revealed that 4 out of 5 of the gray colonies had established and produced apparently normal young. In every case, green spots were present on the leaves. The pink colonies also had reproduced, but in not a single case were there any green spots.

Further field observations showed gray individuals present in all colonies found on green-spotted plants and only pink ones where no green spotting was evident. It is almost invariably true that the typical pink forms greatly outnumber the gray on any one plant. Colonies grown on a single plant for many months may show no green spotting on the bases of the new leaves, even though all the old leaves and the upper portions of the central whorl may be literally covered with green spots. This indicates fluctuation within the colony. On transfer of these mealy bugs to a new plant, green spots may appear, indicating either a change in the status of the individuals transferred, possibly stimulated by the fresh plant, or the development of the green-spotting capacity by the progeny.

Attention was then turned to approximately 160 colonies growing on seedlings in the greenhouse. These were from a great many sources; from pineapples from the various islands of Hawaii and from a number of wild hosts that were being tested as reservoirs of green spot. An examination showed that in all but 5 cases the association of the gray form with green-spotting colonies held true. In these 5 cases the colony was either absent or very small. No gray forms were encountered in any of the nongreen-spotting colonies.

Preliminary rearing tests show that the progenies of gray individuals are all normally pink at first and that some of these will produce green spots, while others will not. The gray forms do not assume that aspect until they are half-grown or even later.

The data indicate some interesting lines of investigation. The rearing of long series of progenies from both forms, with records of each individual

with respect to color and green spotting, is necessary to establish whether or not the gray form is a genetic expression or whether it is indicative of a physiological state of the colony or both.

The facts that it is possible to differentiate colonies by a macroscopic difference in body color and that these colony differences are associated with such profound variations in secretion of the insects as green spotting and nongreen spotting seem to indicate are of great significance in studies of the nature of the toxicity of insect secretions to plants.

DISCUSSION, WITH PRELIMINARY EVIDENCE, ON A HYPOTHESIS CONCERNING A POSSIBLE FUNCTION OF THE SYMBIONTS OF PSEUDOCOCCUS BREVIPES

There appear to be two separate and distinct localized effects of the feeding of *Pseudococcus brevipes* on pineapple leaves. One of these, the irregular chlorotic area, is the result of rather prolonged feeding, usually by a number of individuals feeding at one place. Its general character and gross anatomy suggest that it is the result of exhaustion of the cell contents. Its area does not appear to extend much beyond the actual area fed upon. It is closely allied to spotting caused by the feeding of other Coccidae, such as *Diaspis bromeliae* Kern. and *Pseudococcus longispinus* Targ., and probably represents the normal type of mealy-bug feeding injury. The green spot is clearly in quite a different category. It appears to have a definite developmental history, beginning as a zone of hyperactivity, continuing as an area in which the chloroplasts are larger and more numerous, and ending as a welt-like simulation of a gall. Its zonate character indicates diffusion, perhaps of specific secretion components, outward from the darker center of the spot. The fact that frequently this outer zone of paler green is lacking indicates the possibility of variations in the character of the secretion, even in green-spotting individuals. When one considers the colony pedigrees as shown in figures 4 and 5, B, it is evident that the capacity to produce green spots is clearly specific to certain colonies when these colonies are considered as units. When individual mealy bugs are tested, it is shown that not all the members of a green-spotting colony can produce green spots at any one time. Certain individuals, distinguishable on account of their distinctly brown body color, which produces a grayish cast to the waxy covering, are regularly able to produce green spots. Their progeny, at first normal pink in color, are not all able to produce green spots, though some have this capacity. It is not yet known whether or not all those producing green spots develop the atypical grayish cast. The evidence is clear, however, that there are great variations, even within the progeny of a single female.

If the chlorotic spot is the normal type of mealy-bug feeding injury it would seem that green spotting is a special case superimposed on the nor-

mal one. This is suggested by the iodine-staining reaction of green-spotted tissue (Fig. 2, C), and the disappearance of the intense green, leaving a chlorotic spot in place of the dark center (Fig. 1, C). It would seem also that green spots are formed only normally either at or very close to the white tissue at the proximal end of the leaf. When green-spotting mealy bugs are caged on the older tissue, chlorotic spots result.

The mealy bug, though possessed of functional legs throughout life, and ambulatory at will, is, nevertheless, a sedentary insect. If undisturbed it will, under normal conditions, remain at one feeding point for most of its life. A green-spotting mealy bug, on the other hand, appears unable to remain long in one place, a fact suggesting that the secretion rapidly renders the area unsuitable for further feeding. It is, nevertheless, true that green-spotting colonies appear to grow more vigorously on pineapple plants than do nongreen-spotting colonies, but no data are available on the relationships between this vigor and the presence of gray individuals.

While negative evidence obtained in mechanical inoculation cannot be considered as final, the evidence on insect transmission is conclusive and of very great interest. The failure of nongreen-spotting mealy bugs to become capable of forming green spots after long feeding on green-spotted tissue, coupled with the fact that larvae of a green-spotting parent are able to produce green spots on the first leaf on which they feed, is evidence that green spotting is not the result of infection of the plant by a specific organism but is, rather, a response by the plant to a secretion of the mealy bug, which is specific to certain individuals and not common to the species.

The secretion is, no doubt, the end result of the operation of some internal factor on the physiology of the insect. The hereditary nature of this factor, however, leads to a consideration of the insect's symbionts as being concerned. The presence of symbionts in more or less highly developed mycetomes or "symbiotic organs" in Coccidae has been known for many years and a rich literature on the subject is available. As far as the writer is aware, no suggestion as to the possible relationship between these organisms and the secretions of the host insect, particularly with reference to pathological effects on plants, has been made.[2] For that reason, the following preliminary evidence is presented, since it offers some support to the working hypothesis that the activities of the symbionts affect the insect's secretions.

[2] Rambousek's suggestion (5) that the mycetome plays a definite part in the spread of virus diseases is interesting but difficult to reconcile with the fact that the Homoptera as a group possess mycetomes with included symbionts, irrespective of whether they transmit virus diseases or not. Since that author's paper appeared, the transmission of virus diseases by thrips has been shown by several workers. This fact further negatives Rambousek's suggestion.

All the individuals of the species possess symbionts contained in organized mycetomes. With respect to green spotting, therefore, a fundamental premise must be that either the insect is disymbiotic or the symbionts belong to a single polymorphic species, this polymorphism being paralleled by variations in the physiological effects.

Comparison of the mycetomes of gray and pink mealy bugs, particularly when the pink forms are obtained from a colony in which no gray forms are present, reveals differences that can readily be seen with the naked eye. The mycetomes from definitely gray forms are brown, while those from pink forms present the normal appearance of freshly dissected white tissue. Under low-power magnification the brown mycetomes appear denser and with a more clearly defined membrane. They are less easily damaged in dissection.

Comparison of a long series indicates that the development of this type of mycetome is progressive and all stages can be found in a mixed colony of pink and gray forms, great variation being encountered in mycetomes from individuals determined by inspection as "pink."

Study of the included organisms reveals a common type and, in addition, a second form whose incidence varies according to the status of the colony with respect to green spotting. This second type of organism is bacterium-like in form. It is found intracellularly in the mycetocytes. Thus far, it has been possible to consistently demonstrate its presence in large numbers in the mycetocytes of gray forms; rarely, in pink forms in a mixed colony. On 2 occasions only, in colonies from pineapple plants that had a record of not producing green spots, were these organisms found. Other colonies of this type, as well as those direct from red-top Natal grass, have been consistently negative.

There are some curious parallelisms between green spotting and mealy-bug wilt that should be mentioned. It has been shown (1) that mealy bugs vary in their capacity to produce wilt according to the host plant from which they are taken, those from the roots of red-top Natal and panicum grasses being least toxic.

Direct transfer of mealy bugs from these 2 grasses has thus far failed to demonstrate green spotting. There are evidences of fluctuations in toxicity of mealy bugs according to the age and condition of the plants from which they are taken, while a progressive development of green spots both in size and type occurs. A degree of parallelism between these last two is thus indicated. When the plants accommodate a very large population of mealy bugs, green spots of type 5 are commonly encountered.

The progressive development of the mycetome and the incidence of the bacterium-like inclusions suggest an angle of approach to the question of the parallelism between green spotting and mealy-bug wilt.

CONCLUSIONS

Pseudococcus brevipes causes 2 general types of spotting on pineapple leaves. One is the chlorotic area commonly associated with Coccid feeding. The other is an entirely different type known as green spot. This assumes many forms. It has a distinct developmental history. Colonies of mealy bugs have been maintained for long periods with no green spotting occurring; for similar periods with the constant occurrence of green spots; or a colony may produce green spots at intervals.

Mealy bugs from nongreen-spotting colonies have failed to produce green spots after feeding on green-spotted tissue.

The ability to produce green spots is transmitted from the mother to her young and is limited to certain individuals in the colony. Not all the progeny of such individuals can produce green spots.

Preliminary evidence justifies the working hypothesis that the insect's secretions are conditioned by the activities of the mycetome and its included symbionts.

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THERMAL DEATH POINT OF HETERODERA RADICICOLA IN RELATION TO TIME[1]

HELENE MORITA HOSHINO AND G. H. GODFREY

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Recent investigations on the environmental relations of the root-knot nematode *Heterodera radicicola* (Greeff) Müller[2] at this station include studies on the relations of moisture, light, and temperature to this organism in its different stages. The studies of moisture and light relations are reported in another paper (13). The present paper covers in detail the investigations with controlled temperatures, given in part in a brief preliminary report (12).

There is reported in the literature very little exact research on this phase of the environmental relations of *Heterodera radicicola*. Stone and Smith (15) gave 60° C. as the soil temperature necessary to kill the nematode but stated that for practical purposes it would be advisable to use 82° to 100° C. because of the irregularity of heat penetration in large areas of soil. Bessey (2) concluded that heat was the most efficient means of eradicating nematodes from the soil. Garman (10) reported that dry heat and steam were satisfactory agents for disinfecting soil infested with carnation-root-knot nematodes. Cobb (6) recommended the use of steam. Frandsen (9) stated that at 40° C. every egg and larva was killed at the end of 18 hours. Beinhart (1) reported the need for maintaining a soil temperature of from 72° to 100° C. for ½ hour to sterilize seed beds. Melchers (14) stated that a temperature between 88° and 93½° C. should be maintained for at least ½ hour for controlling nematodes. Byars and Gilbert (5), in connection with hot-water sterilization, reported that, for effective killing of nematodes, a soil temperature of about 55° C. must be maintained for 30 to 60 minutes.

Duruz (7), investigating the effects of temperature on the activity of this nematode, reported greatest activity between 18½° and 29½° C. and that larvae and eggs were killed at 38½° C. Godfrey (11) reported results on the effects of temperature on the development of root knot. Tyler (16)

[1] Published with the approval of the Director as Technical Paper No. 38 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Since this paper has been in form for publication the following paper has been received: GOODEY, T. On the nomenclature of the root-gall nematodes. Jour. Helminth. 10: 21–28. 1932. Goodey presents with convincing evidence the invalidity of *radicicola* as a specific name for this organism. The writers prefer to wait, however, until possible questions as to the validity of the suggested new name are settled before adopting any change in nomenclature.

reported on the effects of temperature on the length of life history. There is no report of exact work on time and lethal-temperature relations.

METHODS

In the tests for determining the lethal temperatures for the larvae and eggs moisture and light conditions favorable to life were maintained. The apparatus used was a thermostat-controlled water bath with a turbine stirrer. A standardized thermometer graduated to 0.1° C. was kept at a constant level in the bath for temperature observations. Pyrex-glass test-tubes, 70 mm. long by 18 mm. in diameter, capacity about 2 cc., containing water up to 10 mm. from the brim, were suspended in the bath by test-tube holders. The temperature of the water in these tubes was checked as to equilibrium, with that in the bath, for each new adjustment. The larvae or eggs were inserted into the tubes in a small drop of water by means of a pipette. Temperatures were not reduced more than 0.1° C. by this process. Temperature fluctuations were never greater than 0.5°. At the end of each period of exposure the vials were removed and immediately placed in a bath of ice water in order to prevent further action of the heat on the organisms. The drop to room temperature took place in a few seconds. The possible effect of the sudden drop in temperature brought about by this method has been considered. The writers feel that this has not been a factor in the killing. Repeated tests were made in which larvae were changed from room temperature (22° C.) to actual freezing in a few seconds, and back to room temperature again, without damage to the nematodes. Other tests, in which lethal temperatures for very short (distinctly nonlethal) periods were tried, likewise showed no injurious effect.

Examinations of larvae were made within a few hours, after each exposure to controlled temperature, and results recorded. Further examinations were made at intervals to determine if apparent killing was only temporary injury. Eggs were removed and incubated according to standard procedure. Controls were kept under favorable moisture, temperature, and light conditions by placing them in a drop of tap water on a slide on a moist filter paper in a Petri dish, which was left in the laboratory. Readings on these were taken at the same time as those for exposed organisms.

The usual 10-minute exposure used in bacteriology was given in preliminary tests. In addition, larvae and egg masses were subjected to a graduated range of higher and lower temperatures, and the minimum period for killing was determined for each. One hundred newly hatched, active larvae were employed in every case in which larvae were tested; 2 egg masses were used in the case of the eggs. Each test with larvae was repeated 5 times and each one with egg masses 6 times. Results were very consistent. Averages only are reported.

EXPERIMENTAL

The results of the several tests on time and temperature relations are given in compact form in the accompanying tables. Instead of incorporating the figures for all replications, and for the sake of greater compactness, averages only are given without probable errors. The figures on percentage survival for periods shorter than the one that was 100 per cent lethal are remarkably uniform in many cases in the different replications. Furthermore, the averages show a logical trend toward lower percentage survival as the time increases. A more exact statistical analysis of the averages, which would be brought about by calculating probable errors, is not justified by the needs of the case. The intervals of time between readings naturally vary widely at different temperatures. The lower temperatures, at which complete killing required hours, and in the case of egg masses, days, were given much wider intervals than the higher temperatures, in which complete killing was attained in minutes or even in seconds. In the tables the second column is devoted to the time interval between readings, the subsequent column headings being the number of such intervals from the start of exposure. To compute the time necessary for complete killing, then, it is necessary to multiply the interval period by the number of such intervals that have elapsed.

The true period at which complete killing was attained is one-half the interval below the time at which complete killing was first recorded, plus or minus half the interval. Thus in table 1, at 42° C., for example, complete killing was attained at 22.5 minutes, plus or minus 2.5 minutes. Table 1 gives the results obtained from the hot-water treatment of *Heterodera radicicola* larvae, there being 5 replications, 100 larvae to a tube, for each temperature.

Heterodera radicicola larvae have complex protoplasmic organization and probably wide differences in thickness of the cuticle, size of body openings, etc. The result is considerable variation in resistance to heat. An attempt to determine whether or not there is a relatively straight-line relationship between percentage survival and time, when plotted on semilog paper, according to the method of Bigelow (3) with certain bacteria, later used by Esty and Williams (8), is shown in figure 1. At the intermediate temperatures and the intermediate time intervals, it is evident that such relationship exists to some extent. Within limits, such a graph can be used to determine the length of time required to kill this nematode at any temperature within the range of lethal temperatures.

The eggs, as they occur in egg masses, show a much higher resistance to heat than do the larvae. This is shown to advantage in table 2, which is prepared in the same manner as table 1, there being 6 replications of each

TABLE 1.—*Percentage of survival of Heterodera radicicola larvae at different temperatures and different periods of time (average of 5 readings)*

Temperature °C.	Interval between observations	Number of interval after exposure											
		1	2	3	4	5	6	7	8	9	10	11	12
40	15 min.			100.0	100.0	78.8	38.6	23.4	15.2	0.0	0.0		
41	10 "	99.4	14.4	1.6	0.2	0.0	0.0						
42	5 "	100.0	36.2	24.4	1.6	0.0	0.0						
43	1 "			100.0	100.0	99.8	98.6	87.8	0.0	0.0			
44	15 sec.												
45	15 "										100.0	100.0	97.8
46	15 "						100.0	100.0	99.4	98.6	98.2	84.2	62.2
47	15 "					100.0	100.0	99.0	94.2	80.4	53.4	0.2	0.0
48	5 "	100.0	100.0	99.4	99.8	98.4	96.8	95.8	94.8	84.0	56.8	17.8	0.0
49	5 "	100.0	100.0	99.0	98.4	97.4	96.0	94.8	92.0	70.8	51.2	13.2	0.0
50	5 "	100.0	100.0	98.8	93.6	89.8	88.6	85.0	7.6	6.0	3.4	0.0	0.0
51	1 "					14.0	5.0	0.0					
52	1 "	24.0	0.0										
53	1 "	0.0											

Temperature, °C.	Interval between observations	Number of interval after exposure												
		13	14	15	16	17	18	19	20	21	22	23	24	25
40	15 min.													
41	10 "													
42	5 "													
43	1 "													
44	15 sec.							100.0	100.0	98.4	79.4	20.0	0.0	0.0
45	15 "	92.8	88.2	82.2	61.4	41.2	32.4	20.4	0.0					
46	15 "	22.2	5.6	0.2	0.0	0.0								
47	15 "	0.0												
48	5 "	0.0												
49	5 "	0.0												
50	5 "													
51	1 "													
52	1 "													
53	1 "													

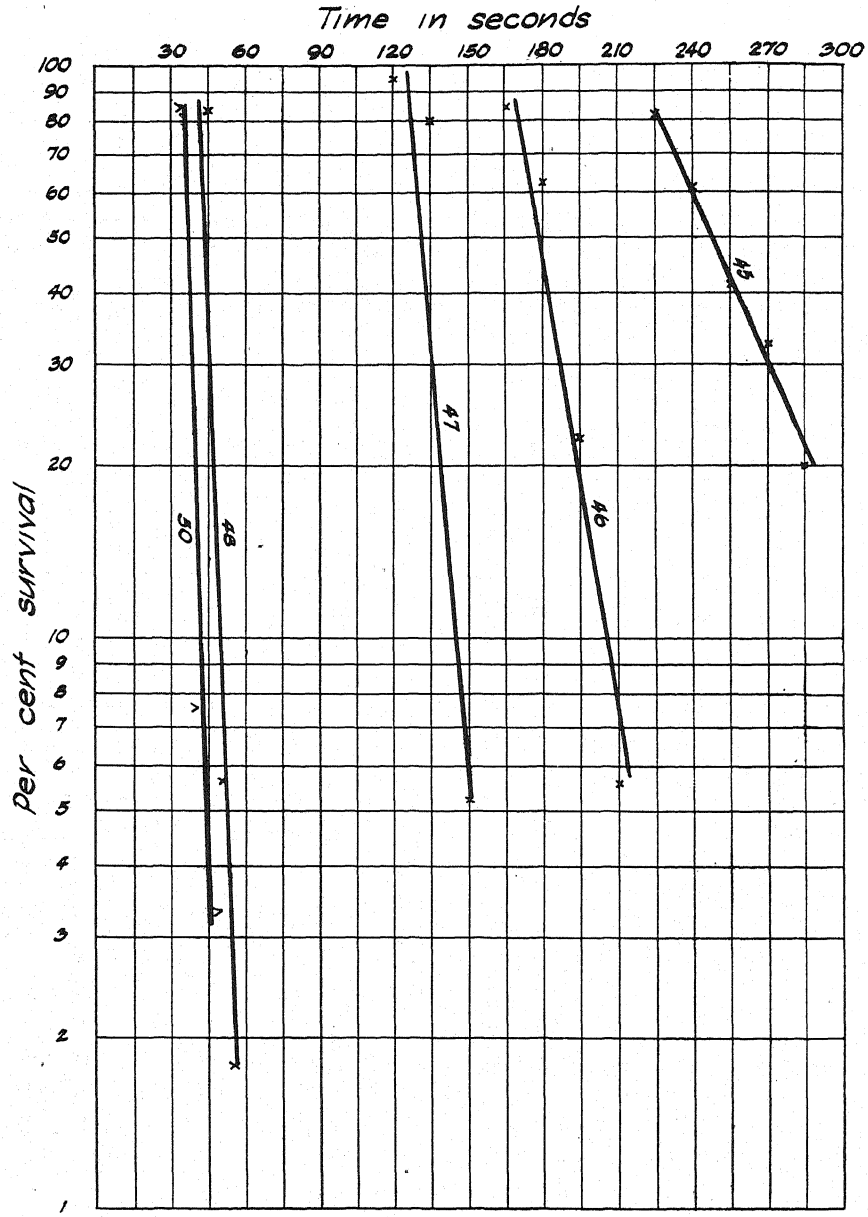


FIG. 1. Semilog graph with survival curves of *Heterodera radicicola* larvae at 45°, 46°, 47°, 48°, and 50° C. While the high ends of the curves are somewhat irregular, the intermediate points show a striking straight-line relationship.

temperature treatment, 2 egg masses in each tube. The averages of the 6 repetitions only, without probable error, are here recorded as being sufficiently accurate for all needs. For heat treatments designed for complete killing of nematodes the 0.0 per cent survival temperatures are, of course, the significant ones, such temperatures being required for complete efficiency in killing. At 40° C., the lowest lethal temperature for both larvae and eggs, 4.5 days (± 0.5 day) were required to kill all the eggs, as compared with only 127.5 minutes (± 7.5 minutes) in the case of larvae. Other temperatures are in proportion. At 50° C., complete killing occurs at 3.5 minutes, ± 0.5 minute. There was instantaneous killing at 58° C., with killing at 57° in 3 seconds and at 56° in 5 seconds. These last figures are only approximate because of the human equation, but they are clearly sufficiently accurate for all practical purposes.

No attempt is here made to show the log-graph relationship between time and percentage of egg survival. It is certain from a few trials that irregularities were considerably greater than with larvae.

The data on "thermal death times" of eggs in egg masses are plotted according to the method of Bigelow (3) on semilog paper, temperatures against time, and show a remarkable straight-line relationship, except at the higher and lower temperatures, as shown by figure 2. The curves are so drawn as to pass between the last positive for survival, represented by a cross, and the first negative, by a circle, in the greatest possible number of pairs of observation points. It is to be noted that, contrary to the results recorded by Bigelow with spore-forming bacteria, the curve turns downward at the higher temperatures, showing a tendency for quicker killing than would be indicated if the straight line of the intermediate temperatures were followed. Likewise, contrary to Bigelow's curves with bacteria, the curve turns upward at the lower temperatures, showing a tendency toward slower killing than would be expected from the continuation of the straight line of the intermediate temperatures. All points above the straight line represent, of course, time and temperature combinations that are fatal to the stage of the nematode indicated, and all points below represent combinations at which the organism survives.

Figure 2 also presents similarly the data with *Heterodera radicicola* larvae. Here the straight-line relationship strictly holds only for temperatures between 43° and 47° C. and the times between 3 and 8 minutes. At higher temperatures the killing is quicker and at lower temperatures slower than indicated by the continuation of the straight line of the intermediate temperatures. At the lower temperatures, between 40° and 43° C. another straight line exists, connecting all 4 points of observation and showing by its more gradual slope the slower killing than at the intermediate temperatures, with both eggs and larvae.

TABLE 2.—*Percentage survival of Heterodera radicicola eggs in egg masses at different temperatures and different periods of time (average of 6 readings)*

Temperature, °C.	Interval between observations	Number of interval after exposure										
		1	2	3	4	5	6	7	8	9	10	11
40	6 hr.	98.65	98.68	73.99	62.79				15.26 (18 hr.)	(24 hr.)	(25 hr.)	(26 hr.)
41	1 hr.	98.71	94.50	90.78			82.27	75.72	36.18	21.07	19.71	18.7
42	30 min.	98.8	88.13	51.13	31.90	5.08	1.19	0.0	0.0			
43	10 "	92.26	79.96	67.54	28.53	27.92	22.07	6.88	5.17	2.73	0.0	
44	5 "	94.11	80.93	63.70	30.67	17.0	3.23	2.28	1.14	0.39	0.0	0.0
45	1 "	98.30	98.29	94.45	93.30	90.70	84.24	81.96	79.33	75.58	71.18	35.72
46	1 "	98.07	85.93	69.58	60.96	53.49	43.87	37.48	34.02	25.34	18.48	11.74
47	1 "	93.65	76.64	57.68	51.71	48.55	34.35	22.59	21.68	16.07	11.33	0.0
48	1 "	73.36	57.18	53.0	29.71	17.06	3.29	0.0	0.0			
49	1 "	56.23	43.22	4.93	2.25	0.0	0.0	0.0				
50	1 "	2.68	2.63	0.25	0.0							
51	10 sec.	54.0					1.0					
52	5 "	97.0	74.0	1.0			0.06					
53	5 "	1.0	0.05				0.02			0.0		
54	1 "	95.0				0.0						
55	1 "	80.0				0.0						
56	1 "			12.0	3.7	0.0						
57	1 "		2.9	0.0								
58	1 "	0.0										

TABLE 2.—(*Continued*)

Temperature °C.	Interval between observations	Number of interval after exposure								
		12	13	14	15	16	17	18	19	20
40	6 hr.	2.77 (27 hr.)	(28 hr.)	(29 hr.)	(30 hr.)	0.65 (31 hr.)	(32 hr.)	(33 hr.)	(34 hr.)	0.0 (35 hr.)
41	1 hr.	13.88	11.48	9.33	5.80	3.24	1.45	0.97	0.0	0.0
42	30 min.									
43	10 "									
44	5 "	0.0								
45	1 "	19.47	13.73	4.78	0.0					
46	1 "	6.07	2.50	1.79	0.0					
47	1 "	0.0	0.0							
48	1 "									
49	1 "									
50	1 "									
51	10 sec.	0.0								
52	5 "	0.0								
53	5 "									
54	1 "									
55	1 "									
56	1 "									
57	1 "									
58	1 "									

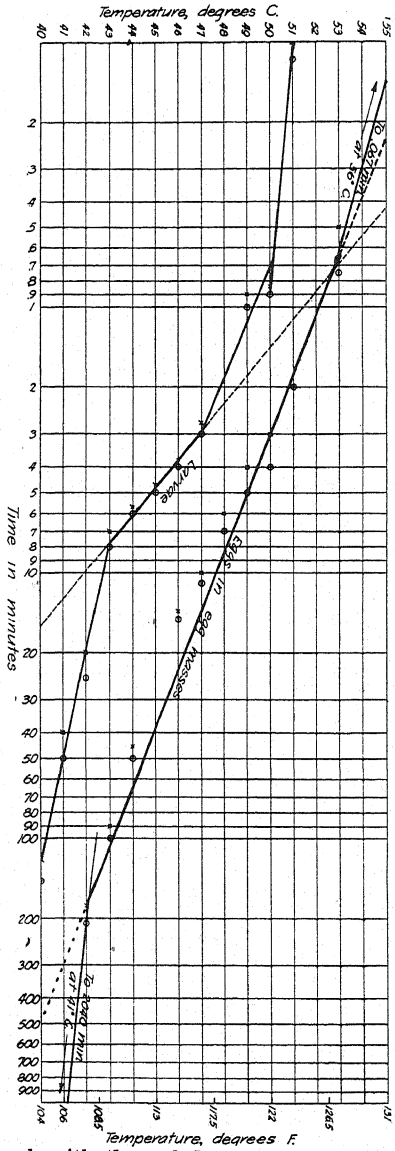


FIG. 2. Semilog graph with thermal death time curves for *Heterodera radicicola* larvae and eggs. Note the relatively straight-line relationship between temperature and time at the intermediate points. All points above the curve represent time and temperature combinations that are fatal to the stage indicated, and all points below represent points at which the nematode survives.

The mathematical theory upon which the use of the semilog paper for thermal death-time curves is based is explained by Buchanan and Fulmer (4, p. 109).

As stated before, special studies were conducted to determine the minimum temperatures at which 10-minute exposures would bring about complete killing, according to practices common in bacteriology. In these studies there were 5 replications of each treatment, 2 egg masses to a tube. Without resorting to tabular form, the results in average survival of larvae for the significant temperatures are as follows: 40° C., 100 per cent; 41° C., 99.4 per cent; 42° C., 36.2 per cent; 43° C., 0 per cent. The lethal point, then, is 42.5° C. ± 0.5°. With egg masses the averages of 6 replications are as follows: 42° C., 99.68 per cent (within the range of the usual controls); 43° C., 92.2 per cent; 44° C., 80.93 per cent; 45° C., 71.18 per cent; 46° C., 18.48 per cent; 47° C., 11.33 per cent; 48° C., 0 per cent. The lethal point is 47.5° C. ± 0.5°, for 10-minute exposure.

CONCLUSIONS AND SUMMARY

This paper constitutes the results of carefully conducted experiments with *Heterodera radicicola* larvae and eggs exposed to direct hot-water treatment at a wide range of temperatures; and, from them, exact data are recorded on the percentage of survival of these stages of the nematode at different temperatures and periods of time and the minimum periods required for complete killing at each of the lethal temperatures. Tables 1 and 2, giving the above information in detail, are here summarized as to the latter point only. The minimum periods of time required for killing the larvae are for the different temperatures in degrees Centigrade: at 40°, 2 hours, 7.5 minutes ± 7.5 minutes; 41°, 45 minutes ± 5 minutes; 42°, 22.5 minutes ± 2.5 minutes; 43°, 7.5 minutes ± 0.5 minute; 44°, 5 minutes, 52.5 seconds ± 7.5 seconds; 45°, 4 minutes, 52.5 seconds ± 7.5 seconds; 46°, 3 minutes, 52.5 seconds ± 7.5 seconds; 47°, 2 minutes, 52.5 seconds ± 7.5 seconds; 48°, 57.5 seconds ± 2.5 seconds; 49°, the same; 50°, 52.5 seconds ± 2.5 seconds; 51°, 6.5 seconds ± 0.5 second; 52°, 1.5 seconds ± 0.5 second; 53°, 1 second. The minimum periods for the eggs are: 40°, 4.5 days ± 0.5 day; 41°, 33.5 hours ± 0.5 hour; 42°, 3.25 hours ± 0.25 hour; 43°, 95 minutes ± 5 minutes; 44°, 47.5 minutes ± 2.5 minutes; 45°, 14.5 minutes ± 0.5 minute; 46°, same; 47°, 10.5 minutes ± 0.5 minute; 48°, 6.5 minutes ± 0.5 minute; 49°, 42.5 minutes ± 0.5 minute; 50°, 3.5 minutes ± 0.5 minute; 51°, 1.5 minutes ± 0.5 minute; 52°, 45 seconds ± 15 seconds; 53°, 37.5 seconds ± 7.5 seconds; 54°, 5 seconds ± 2.5 seconds; 55°, same; 56°, 4.5 seconds ± 0.5 second; 57°, 2.5 seconds ± 0.5 second; 58°, 1 second.

These results are arranged in the form of semilog graphs. The temperatures are plotted on the arithmetic scale and time intervals on the logarithmic. At the intermediate points in time and temperature, a very good straight-line relationship exists. This enables one to see at a glance the

time and temperature combinations at which killing occurs and those at which some of the nematodes will survive.

These temperatures and time periods are exact for the killing of the 2 stages of the nematode. It is necessary, of course, that the specified temperatures reach the organisms and that they be maintained there for the necessary period before the killing is attained. This information is fundamental for any further studies that may involve the application of temperature for root-knot-nematode control. The lethal temperatures are much lower than those commonly employed in practice when it is desired to destroy nematode infestation in the soil or in dormant plant tissues. It will continue to be necessary to employ higher temperatures than those specified in order to obtain penetration of the necessary temperatures to the points in the soil or other medium in which the nematodes are actually located.

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THE TOXICITY OF FORMALDEHYDE AND MERCURIC CHLORIDE SOLUTIONS ON VARIOUS SIZES OF SCLEROTIA OF RHIZOCTONIA SOLANI[1]

G. B. SANFORD AND J. W. MARRITT[2]

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During recent years several papers (2, 3, 4, 5) have appeared on the relative fungicidal efficiency of various chemicals used in solution for reducing the Rhizoctonia disease of potatoes. The relative superiority of these was based primarily on the amount of sclerotia on the tubers of the new crop produced under ordinary field conditions. Melhus, Gilman, and Kendrick (5), also Gloyer (2), formed their conclusions partly by culturing the treated sclerotia and partly by the amount of sclerotia on the new tubers. Obviously the plate-culture test should settle the relative merits of each treatment in killing the sclerotia. With regard to residual effect from formaldehyde or mercuric chloride solutions in protecting the new plantlet, this would be exceedingly difficult to demonstrate, especially in old cultivated soils.

However, as far as could be ascertained, the relation of the size of the sclerotia to the requisite lethal period has not been studied. This would seem important, inasmuch as the size as well as the texture of these bodies varies greatly under various conditions. They may be friable and easily killed or so compact and hard that successful penetration is greatly retarded.

MATERIAL AND METHODS

The sclerotia were classified into sizes, *viz.*, small, medium, and large, and samples indicating the size range for each class mounted on a chart for guidance. Each size varied in flat diameter and thickness, respectively, as follows: Small, about 1.5 mm. by 0.2–0.4 mm.; medium, about 2.5 mm. by 0.5–0.7 mm.; large, about 3.5 mm. by 0.8–1.5 mm. Extra large or extra small or very fragile sclerotia were avoided as far as possible. The treatments included cold formaldehyde solutions, strengths 1–120, 1–160, 1–200, and 1–240; cold mercuric chloride, strength 1–834, and mercuric chloride, strength 1–500 acidulated, with 1 per cent by volume of hydrochloric acid, as recommended by Leach, Johnson, and Parsons (4).

[1] A contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, cooperating with the Department of Field Crops, University of Alberta.

[2] Plant Pathologist in Charge and District Potato Inspector, respectively.

Tubers with sclerotia of various sizes were treated, always in a fresh solution, without preliminary wetting or cleaning, for different periods as required, then covered, and allowed to dry. The drying period required about 3 hours, which is comparable to usual practice on the farm. The sclerotia were then plated on potato-dextrose agar made acid by mixing in the cooling medium, 3 per cent by volume of 5 per cent lactic acid. This allowed the Rhizoctonia fungus to grow satisfactorily but controlled the bacteria. In fact, it was found essential to acidify the agar to do reliable work. Each size of sclerotia was plated separately. Readings were made at intervals up to 4 days, which time was sufficient to indicate whether the bodies were killed.

The sclerotia were found by repeated tests to be almost 100 per cent viable, thus making unnecessary controls for each treatment. Moreover, it was found that the growth of bacteria and molds from untreated sclerotia produces unsatisfactory results. The mercuric chloride and hydrochloric acid used was "Bakers C.P. analysed." The formalin was a commercial brand found by chemical analysis in our laboratory to contain 37.67 per cent formaldehyde. The results throughout are given to the nearest decimal. The relative humidity of the room in which the potatoes for the formaldehyde tests were stored was about 85 per cent and that of the room in which the stock for the mercuric chloride tests was kept, about 56 per cent.

FORMALDEHYDE AND MERCURIC CHLORIDE TREATMENT

Formaldehyde. The results in table 1 and figure 1 indicate the general trend of the lethal period required for the small, medium, and large sclerotia in the 4 strengths of cold formaldehyde solution mentioned. Although time intervals of 10 minutes were employed, only the results at 30-minute periods are given in the table. At strength 1–120, all the small sclerotia were killed in 90 minutes, the midsize ones, in 180 minutes, and those of large size, in 270 minutes. At strength 1–160, the lethal periods for the small, mid-, and large sizes were 120, 240, and 360 minutes, respectively, and in strength 1–200 were 120, 300, and 400 minutes, respectively. In strength 1–240, the small bodies were all dead in 150 minutes, the midsize ones in 390 minutes, while about 1 per cent of the large size were still viable after being immersed for 480 minutes. Further, it should be noted that in a strength of 1–240, 19 per cent of the midsize bodies and 56 per cent of the large size were viable at the end of 2 hours. Apparently cold formaldehyde, strength 1–240 (1 pt. to 30 gal.) is effective only for small sclerotia or where tubers are relatively clean. It is felt that these results would apply to average sclerotia as found in nature in Alberta, although evidence obtained during these studies indicates quite conclusively that the sclerotia

TABLE 1.—*The percentage*[a] *of small, medium, and large sclerotia of Rhizoctonia solani viable after treatment in various strengths*[b] *of formaldehyde solution for various periods at 20–25° C.*

Time[c]	Small				Midsize				Large			
	1/120	1/160	1/200	1/240	1/120	1/160	1/200	1/240	1/120	1/160	1/200	1/240
30	2	6	14	21	26	35	54	67	43	57	93	
60	1	2	19	13	16	20	30	42	30	48	66	
90	0	1	3	5	8	14	18	27	26		40	70
120		0	0	2	3	10	13	19	22		31	56
150				0	1	7	9	17	16	19	23	42
180					0	5	7	12	10	13		31
210						2	5		5	8		
240						0	2		2	6		23
270							2	5	0	4	10	20
300							1	3		3		
330							0	2		2	5	9
360								1		0		
390								0			3	5
420											1	3
450											0	2
480												1
510												0

[a] Based on 200–400 sclerotia for small size, 100–150 medium size, and 50–90 sclerotia for large size, and verified by treatments made at intermediate periods of 10 minutes.

[b] Indicated by 1/120, 1/160, 1/200, and 1/240.

[c] Minutes immersed in solution (not presoaked).

on certain lots of potatoes may be harder to kill than those used in these tests.

Melhus, Gilman, and Kendrick (5) obtained 6 per cent of viable sclerotia after treatment for 2 hours in strength 1–120 cold formaldehyde at 26° C. The number of sclerotia used was 100, but no reference was made regarding size or texture of these. At 25° C. and the same time, but strength 1–240, 8 out of 100 sclerotia grew, while, in another lot of 30 planted, none grew. In view of our work, it would seem that their results might be explained partly on the basis of size and texture. It is interesting also to note that Gloyer (2), using cold formaldehyde solution, strength 1–240, for 2 hours, at 17–22° C., obtained 23.8 per cent viable sclerotia out of 327 cultured, and in strength 1–60 at 21° C., for 2 hours, 5 per cent of 60 sclerotia grew. These results are more comparable to those presented in table 1, although Gloyer did not state the size of the sclerotia.

Mercuric chloride. The results from the cold mercuric chloride solution, nonacidulated, strength 1–834, and from the acidulated solutions, strengths

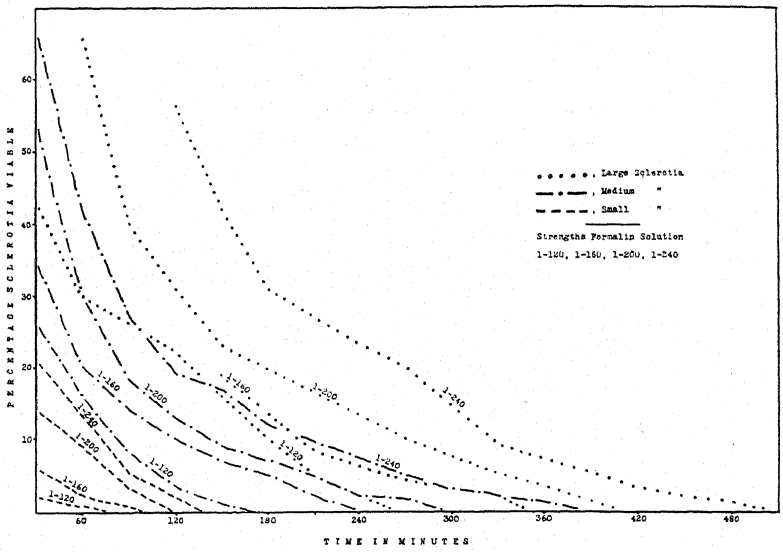


FIG. 1. Viability of small, medium, and large sclerotia of *Rhizoctonia solani* after immersion in formalin solutions of different strengths for various periods.

TABLE 2.—*The percentage of sclerotia of Rhizoctonia solani viable after treatment in solutions of mercuric chloride (acid and nonacid) for various periods at 20–25° C.*

Size	Small				Midsize				Large			
Strengths	1–500		1–834		1–500		1–834		1–500		1–834	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Time[b]	(Acidulated[a] solution)											
3	100	0	100	2	100	1	50	28	28	11	25	68
5	100	0	100	0	100	0	50	6	60	8	30	37
8	100	0	100	0	100	0	40	12	69	6	20	10
10							50	7	81	3	33	33
13							60	3	90	2	25	28
15							50	0	65	0	25	20
	(Nonacidulated solution)											
30			150	5			150	37			84	68
45			190	2			33	14			18	62
60			300	0			57	6			49	43
90			200	0			100	3			60	33
120							150	1			80	25
130							135	0			45	16
150							190	0			71	9

[a] By adding 1 per cent by volume hydrochloric acid C.P.

[b] Minutes in solution.

1–500 and 1–834, are presented in table 2. Apparently the cold mercuric chloride solution, nonacidulated, and strength 1–834, is about as effective as the cold formaldehyde solution, strength 1–120. All the small sclerotia were killed in 60 minutes, the midsize ones in 130 minutes, but 9 per cent of the large sclerotia were still viable after 150 minutes. The end point was not determined, but it would likely be about 200 minutes. Thus, where large sclerotia are very scarce, a treatment for 2 hours or even 90 minutes would be effective for practical purposes, but, where they are numerous, between 25 and 30 per cent would still be viable.

Mercuric chloride, acidulated. Cunningham (1), in New Zealand, first discovered that the addition of hydrochloric acid to mercuric chloride solution reduced the time required to kill sclerotia of *Rhizoctonia solani* to about 5 minutes. Leach, Johnson, and Parson (4), as a result of recent work, recommend treatment for 5 minutes in strength 1–500, plus 1 per cent by volume of hydrochloric acid. Considering the results from the acidulated mercuric chloride treatments (Table 2), at strength 1–834 for 5 minutes, none of the small, 6 per cent of the midsize sclerotia and 37 per cent of the large ones grew. After a treatment of 15 minutes, 20 per cent of the large bodies were still viable. The end point for this size was not determined. At strength 1–500, all the small and medium sclerotia were killed in 5 minutes, but 8 per cent of the large ones grew. Fifteen minutes were required to kill all the large ones. Thus, the time strength of acid mercuric chloride (1–500 for 5 minutes), recommended by Leach *et al.*, would be effective for practical purposes, provided large sclerotia were not abundant and that these were not unusually hard to kill. Further, the striking difference in effectiveness between strengths 1–500 and 1–834 emphasizes the advantage of using the former strength instead of the latter one.

Decrease in strength. Leach *et al.*, (4) determined by chemical analysis the loss in hydrochloric acid and mercuric chloride from the solution (strength 1–500 plus 1 per cent HCl) following successive treatments of potatoes. The original solution had 0.2266 per cent of $HgCl_2$ and 0.376 per cent of HCl. After 8 treatments these were 0.1864 and 0.328 per cent, respectively.

It seemed advisable to determine the decrease in effectiveness by culturing the sclerotia from successive lots treated in the same solution. Equal quantities by weight of potatoes bearing sclerotia were immersed, one after the other, in the same solution for the required time (5 minutes for each lot in the acidulated solution or 60 minutes in the nonacidulated one) and the sclerotia plated according to size. Two different lots of potatoes were used for this test: one, lot "A" (same as used throughout this study), from which only the small sclerotia were cultured; and another, lot "B,"

from which all sizes were plated. The sclerotia from lot "B" were firmer in texture and, as indicated by the results in table 3, somewhat harder to kill than those of lot "A."

TABLE 3.—*The decrease in effectiveness of solutions of mercuric chloride as indicated by percentage*[a] *of sclerotia of Rhizoctonia solani viable after successive treatments*

	Acidulated—5 minutes				Nonacidulated—60 minutes			
Size	Small		Midsize	Large	Small		Midsize	Large
Lot[b]	A	B	B	B	A	B	B	B
Times	1–834	1–500	1–500	1–500	1–834	1–834	1–834	1–834
1	0	0	2	17	0	3	21	62
2	0	0			0			
3	0	0			0			
4	0	0			0			
5	0	2	4	15	1	3	36	73
6	1	1	4	18	1	5	43	77
7	1	4	5	25	3	10	41	77

[a] Based on 100 sclerotia for small and medium sizes and on 50 for large size.

[b] Lot "A," material as used throughout the study. Lot "B," special material, sclerotia firmer than on lot "A."

In the nonacidulated solution, strength 1–834, for 60 minutes, all the small sclerotia on lot "A" were killed up to the 5th, 6th and 7th treatments, in which 1, 1, and 3 per cent, respectively, were viable. On lot "B," 3 per cent of this size grew after the 1st treatment and 5 per cent after the 6th one. At the same strength, 21 per cent of the medium size grew after the 1st treatment, 36, 43, and 41 per cent after the 5th, 6th, and 7th treatments, respectively; while 62 per cent of large sclerotia grew after the 1st treatment and 73, 77, and 77 per cent after the 5th, 6th, and 7th, respectively.

With regard to the acidulated solution, the small sclerotia of lot "A," in strength 1–834, were all killed up to the 6th and 7th times, when 1 per cent grew in each case. The small sclerotia of lot "B," in strength 1–500, were all killed up to the 5th time, when 2 per cent were viable. Two per cent of the medium size of lot "B" grew after the 1st treatment in strength 1–500 and 4, 4, and 5 per cent after the 5th, 6th, and 7th times. As for the large size, 17 per cent of these grew after the 1st time and 15, 18, and 25

per cent after the 5th, 6th, and 7th times. If we may judge on the basis of results obtained from the small sclerotia, indications are quite definite that either the acidulated or the nonacidulated solution may be used at least 5 times without serious reduction in effectiveness.

EFFECT OF TREATMENT ON VITALITY OF SET

Field and laboratory tests were made of the effect of various treatments, mentioned in tables 1 and 2, on the vitality of the set. Part of a lot of Netted Gem potatoes was kept in cold storage so that only very short sprouts started. On the other part, kept in a basement, sprouts upwards of ½ in. long developed. This material was treated as required, ½ of each tuber being planted in sand in the greenhouse and kept moist, the other half in rows in the field, 20 sets per row, 18 in. apart. The following time-strength treatments of formaldehyde were used: 1–120 for 60, 180, and 240 minutes; 1–160 for 80, 220, and 310 minutes; 1–200 for 100, 270, and 390 minutes; and 1–240 for 120, 320, and 470 minutes.

Using the same procedure, the following mercuric chloride treatments were made: nonacidulated, strength 1–834 for 60, 90, and 120 minutes; and acidulated, strength 1–834 for 5, 10, and 15 minutes, and strength 1–500 for 3, 5, and 8 minutes.

Notes on the greenhouse material were taken at emergence and again when the sprouts were about 6 in. high and on the field material at emergence and at blossom time. Briefly stated, the results in all cases, based on observation, were that no detrimental effects of the various treatments on the vitality of the sets could be seen. Yield data taken also showed no significant differences arising from any of the treatments. Obviously, final conclusions cannot be drawn from only 1 year's results.

SIZE OF SCLEROTIA IN NATURE

The results already presented conclusively show that, in determining the effectiveness of any treatment, size of sclerotia is quite as important as time of immersion or strength of the solution. Hence, it is at least interesting to know the proportion of each size commonly occurring in nature. The data in table 4 suggest the percentage of large, medium, and small sclerotia found on 9 representative lots of potatoes grown near Edmonton in 1931, all of which were harvested rather late from soils of relatively high moisture content. If the scurf could have been counted or estimated as "small" on a numerical basis, the percentage of this size would have been increased. It, however, was found that the proportion of small, medium, and large size, as counted, was roughly 13:3:1. Of course, these figures

TABLE 4.—*Relative sizes*[a] *of sclerotia of Rhizoctonia solani on 9 lots of potatoes, 1931 crop, Edmonton, Canada.*

Lot	Bin[b] rating	Tubers counted	Total sclerotia	Small	Midsize	Large	Number scurfy	Date harvested
	Per ct.			*Per ct.*	*Per ct.*	*Per ct.*		
1 ...	50	29	1,206	83	12	5	15	Oct. 1– 7
2 ...	16	19	419	93	6	1	4	" 5– 7
3 ...	4	28	728	80	15	5	8	" 10–16
4 ...	9	14	272	52	38	10	4	
5 ...	25	34	124	92	8	0	7	Sept. 28–30
6 ...	4	20	475	78	17	5	3	Oct. 1
7 ...		24	658	67	22	11	0	
8 ...	1	11	125	73	21	6	1	Oct. 1– 8
9 ...	6	31	725	69	20	11	0	Sept. 23–30
			Average	76.3	17.6	6		

[a] Dimensions of small, midsize, and large approximately the same as used throughout this paper.

[b] The number of tubers out of 200 chosen at random on which any sclerotia occurred.

are only suggestive, but, if further investigation shows this proportion to be approximately normal, due weight can be given to any of the "time-strength-size" treatments recommended for general use.

DISCUSSION

These studies show very clearly that the standard cold formaldehyde solution (1–240 for 2 hours) is not effective for general use in treating tubers where medium and large sclerotia are to be killed. Briefly stated, this treatment would not seem justified on any grounds except when sclerotia are practically absent or limited to very thin scurf. Why a number of the bodies were not killed even after 4–5 hours' soaking was not definitely determined, except that they were always the compact, hard ones. Firm, small sclerotia, difficult to kill, were not uncommon. A fairly large proportion of both medium and large sizes were loose in texture and quickly killed. Had the material been presoaked, the effectiveness of all treatments would have been increased materially, but this was not done beyond determining that presoaking is valuable in this connection.

With regard to mercuric chloride, these studies show quite conclusively that a solution of strength 1–834 for 1 hour was about as effective in killing

the small and medium sizes as formaldehyde, strength 1–120 for 2 hours. By adding 1 per cent, by volume, of hydrochloric acid to a mercuric chloride solution, strength 1–500, effective results for practical purposes can be obtained in about 5 minutes, provided large or extra hard sclerotia are not abundant. This fact should be sufficient to recommend its use in place of either the cold formaldehyde or the nonacidulated mercuric chloride solutions for killing sclerotia of *Rhizoctonia solani.*

SUMMARY

The relative effectiveness of various time strengths of cold formaldehyde, nonacidulated, and acidulated mercuric chloride in killing small, medium, and large sclerotia of *Rhizoctonia solani* on the surface of potatoes was determined. The approximate dimensions of these bodies in flat diameter and thickness are given.

In the standard cold formaldehyde solution, strength 1–240, 2 per cent of the sclerotia of small size, 19 per cent of medium size, and 56 per cent of the large size were viable after being immersed for 2 hours. It required 390 minutes to kill all the medium sclerotia and 480 minutes for the large ones. Stronger solutions were increasingly effective for all sizes. The lethal period for the small, medium, and large sizes in strength 1–120 was 90, 180, and 270 minutes, respectively.

The cold mercuric chloride solution, strength 1–834, was more efficient than any of the cold formaldehyde solutions used. The lethal period for the small, medium, and large sizes was about 60, 130, and 150 minutes, respectively, depending upon the texture.

The acidulated mercuric chloride solution, strength 1–500 plus 1 per cent by volume of hydrochloric acid, killed all the small sclerotia in 3 minutes and the medium size in 5 minutes. Eight per cent of the large size were still viable after 5 minutes and 2 per cent after 13 minutes. Treatment in strength 1–834 killed the small sclerotia, but a rather high percentage of the medium and large sizes grew.

Evidence from plating treated sclerotia showed that where small or medium sclerotia are concerned, the solution of either acidulated or nonacidulated mercuric chloride was effective up to the 5th successive treatment and, for practical purposes, probably up to the 8th by extending the time.

The variability in texture of the sclerotia on any lot of potatoes or among different lots is often very marked, making necessary different lethal periods in a given solution.

Under the conditions of the test (one season) none of the following time-strength treatments used perceptibly weakened the vitality of the set:

Cold formaldehyde, strength 1–120 up to 240 minutes; 1–160 up to 310 minutes; 1–200 up to 390 minutes; and 1–240 up to 470 minutes; cold mercuric chloride, strength 1–834 up to 120 minutes; and acidulated mercuric chloride, strengths 1–500 and 1–834 up to 8 and 15 minutes, respectively.

From counts made on 9 representative lots of potatoes the proportion of small, medium, and large sclerotia was 13 : 3 : 1.

DOMINION LABORATORY OF PLANT PATHOLOGY,
UNIVERSITY OF ALBERTA, EDMONTON, CANADA.

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INTUMESCENCES ON LEAVES OF EUCALYPTUS CORNUTA, E. COCCIFERA, HIERACIUM VENOSUM, MITCHELLA REPENS, AND THURBERIA THESPESIOIDES[1]

CARL D. LA RUE

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Intumescences have been reported on a number of plants since Sorauer (8) first applied that name to the masses of cells that appear under certain conditions on leaves, twigs, and even on roots. These outgrowths are usually rather small, and, since, in most instances they are probably not greatly detrimental to the plants, they are often overlooked. The number of species known to produce such abnormalities is, however, increasing from year to year. The writer became interested in intumescences on leaves of poplar some years ago (5) and since that time has noticed their appearance on several plants that have not hitherto been known to produce them.

This paper deals with intumescences on leaves of *Eucalyptus cornuta* Labill., *E. coccifera* Hook., *Hieracium venosum* L., *Mitchella repens* L., and *Thurberia thespesioides* A. Gray.

Eucalyptus cornuta. Several species of Eucalyptus have been reported to produce these abnormalities, but *E. cornuta* has not been named among them. A number of plants of this species are growing in the greenhouses of the Botanical Gardens of the University of Michigan, and very small green intumescences have been seen on all of these. They occur on both the petioles and the blades of the leaves but have not been found on any other organs of the plants. They appear on both surfaces of the leaves, and are not, so far as can be determined, more numerous on one surface than on the other. On the petioles they seem to be scattered at random, without reference to upper or lower surface. They have not been found in great abundance on any leaf; at least not as compared with the numbers that have been seen on leaves of poplars, but 50 or 60 may be developed on one surface of a given leaf.

So far as the time of development goes, it can be said with certainty that they appear only on the young leaves before they are half grown and do not increase in number after that time, although they may persist until the leaves have reached full size. Nothing is yet known of the causes of these excrescences, and no experiments have yet been performed to determine them. Pieces of leaves bearing the intumescences were cut out, killed, imbedded, sectioned, and stained in the usual manner. No difficulties of

[1] Paper No. 381 from the Department of Botany, University of Michigan.

technique were encountered, so it is unnecessary to discuss methods in detail.

It was found that some outgrowths were confined to the epidermis and so were scarcely to be detected by the naked eye. The cells of the upper epidermis were involved in some abnormalities; in others the lower epi-

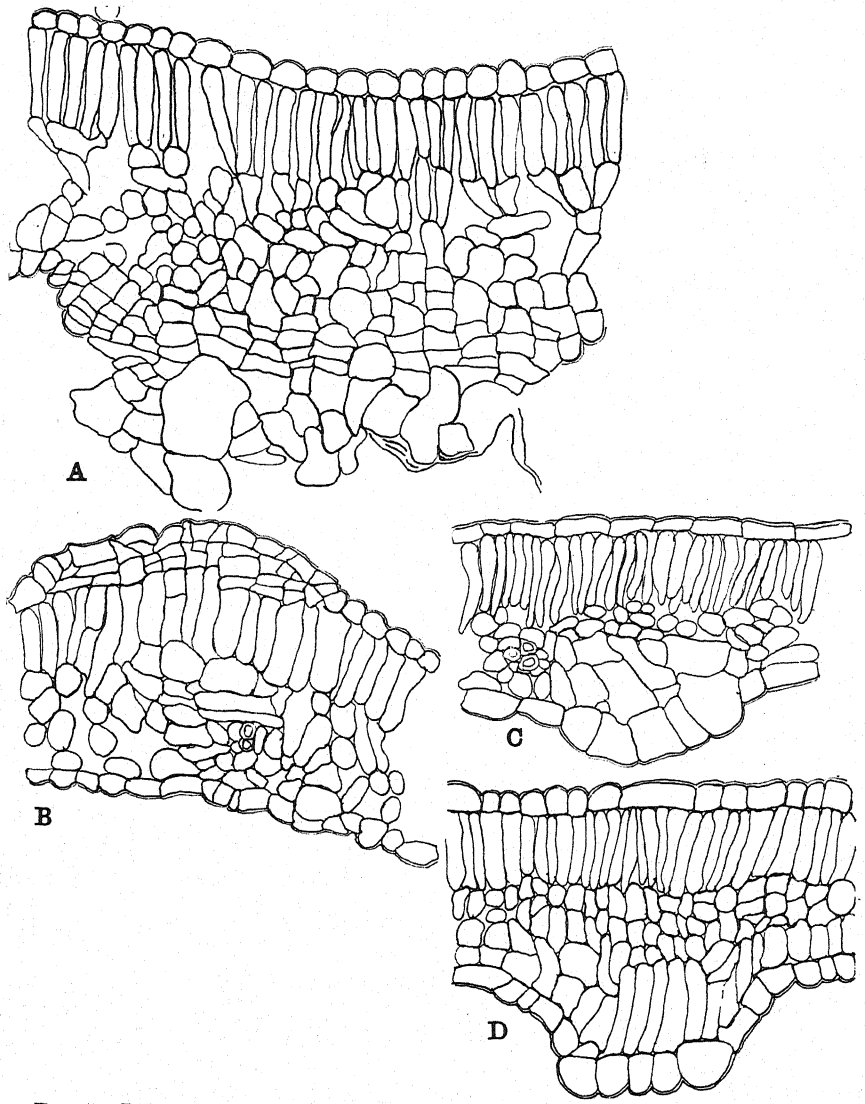


FIG. 1. Intumescences. A. On lower surface of *Eucalytus cornuta*. B. On upper surface of *E. cornuta*. C. Young one on lower surface of *E. cornuta*. D. On lower surface of *Thurberia thespesioides*.

dermis was the seat of the disturbance. Sometimes, the cells are greatly enlarged in all dimensions but have not divided, but, in other instances, they have divided once or twice with a resulting increase in volume.

Outgrowths on the upper surface that extend beyond the epidermis have not been found in any case to reach below the palisade layers (Fig. 1, B). The palisade cells may be elongated without any increase in width and without undergoing division, but, among such cells, others may be seen that have swollen in all dimensions without division, while still others have divided, even repeatedly. Usually, the swollen cells are the ones that divide rather than those that are greatly elongated without corresponding increase in width. The elongation of these cells is, as might be expected, at right angles to the surface of the leaf, since the pressure of other cells on their sides prevents so great an increase in the direction parallel to the surface.

In has been mentioned that the intumescences remain green, and in the sections the chloroplasts are seen to be normal, although their number is not proportional to the increase in volume of the cells. The vacuoles of the cells are greatly enlarged, but no other change in the appearance of the cells could be detected. The cell walls appear to be unchanged except for their increase in extent.

The abnormalities that appear on the lower surfaces of the leaves are quite like those on the upper surfaces, except, of course, for the difference in the tissue involved (Fig. 1, B and C). Here, the changes do not extend beyond the spongy parenchyma but they usually affect all the cells of that tissue. One does not find a great elongation of the cells in one dimension, as occurs in the palisade cells, apparently because the looser tissue allows them to expand more readily in all directions. In this tissue the cells may undergo enlargement without division or they may divide once or more. In other respects the changes are very like those of the palisade cells.

One finds the intumescences, then, whether on the upper or the lower surface, made up of an admixture of enlarged cells, cells without enlargement or division, those that have divided without noticeable increase in size, and others that have divided and also undergone extensive swelling. Such growths might be spoken of as a mixed type, since they are neither hypertrophies nor hyperplasias but a conglomeration of both. In cross section they are seen to vary from 5 to 60 cells in extent.

In many of the intumescences there is a beginning of periderm development, which may have its inception in the epidermis itself, or in any of the layers of the mesophyll, depending on the surface on which the overgrowth is developed (Fig. 1, A). Again, the layer of periderm may form in an arc beginning in the epidermis, on one side, and dipping into the mesophyll to the lower layer of the palisade or to the upper layer of spongy

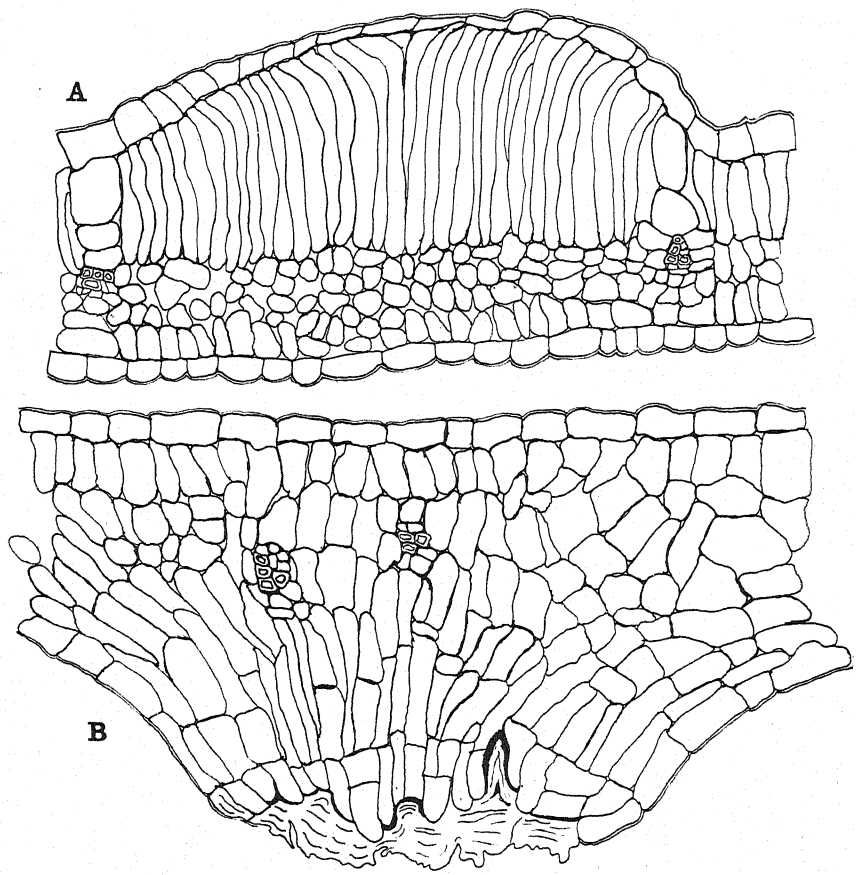


FIG. 2. Intumescences. A. On upper surface of *Thurberia thespesioides*. B. On lower surface of *Hieracium venosum*.

mesophyll, respectively, then passing out to the epidermis, on the other side, so that the intumescence is completely isolated from the remainder of the leaf. Soon after the periderm is formed the cells of the intumescence that lie outside this layer collapse, and the periderm becomes suberized. Thus, the leaf is completely closed against the entrance of fungi and sealed against loss of water.

Eucalyptus coccifera. Sorauer (9) has reported the presence of intumescences in this species but has given no details of their development. Plants of this species, growing alongside those of *E. cornuta* in the Botanical Gardens of the University of Michigan, attracted the attention of the writer because of the presence on the leaves of numerous bright red spots, which were scarcely raised above the surfaces, and did not suggest that they

were of the nature of intumescences. Examination with a microscope, however, indicated such a nature, which was proved by a study of sections of the affected leaves.

The leaves of *Eucalyptus coccifera* are isobilateral, with palisade on both surfaces, although this would not be expected from their position, which is that of ordinary leaves. In general, isobilateral leaves assume a vertical position, and the horizontal arrangement of leaves usually suggests that they are dorsi-ventral as regards their internal structure. In spite of the fact that both sides of the leaves in this species are almost identical, the intumescences are much more abundant on the upper than on the lower surface.

Nothing has been found out as to the cause of these outgrowths, which come near being hyperplasias, since they are made up of cells that are little increased in size over those of the normal leaf. The number of cells, however, is greatly increased in the overgrowths, which, in general, is characteristic of hyperplasias. There is nothing of special note in these growths except the development of periderm, which separates the mature intumescences from the normal parts of the leaf. There is usually one layer of periderm that is very well developed, and that is located in the outer part of the outgrowth. Later on, another layer may be formed near the innermost part of the intumescences, and it is not unusual to find a third layer between these two, which was probably developed before the inner layer. The intumescences that have been studied in this species were mature and were seen to consist of alternate layers of mesophyll cells and periderm. No young stages have been secured for study, so that it is impossible to be sure of the sequence of developments in all cases. All layers of the periderm became suberized.

The bright red of the intumescences seems to depend on the isolation of the mesophyll cells, which contain chlorophyll, from the interior of the leaf, with a consequent accumulation of sugar in the isolated cells, and an extensive development of anthocyanin. The red color indicates an acid reaction in the cells, which has been found by Harvey (1) in intumescences on Ricinus, beet, and Bryophyllum leaves.

Hieracium venosum. The abnormalities on this plant were produced in response to injections of water and of weak solutions of ammonia. Smith (10) found that injections of ammonia and other chemicals caused abnormal growths in *Ricinus communis* L. and other plants. In imitation of his methods, the writer made similar injections into plants of several different species, without result except in *H. venosum.* Some time after the treatment it was found that low intumescences had developed around the site of the injection in a number of the leaves. They appeared both in the

plants injected with ammonia and in those that had received injections of water only. Hence, it appears most likely that the growths are due rather to the injury caused by the insertion of the needle than to the substance injected. All the injections were made through the lower epidermis, and all the intumescences were found on the lower surfaces of the affected leaves and in a ring immediately around each of the wounds.

Sections of intumescences reveal a great increase in cell number in the spongy mesophyll, which is the only tissue affected, if we exclude the epidermis, which is pushed out at first and later is ruptured but not otherwise affected. The cells show considerable swelling and enlargement of the vacuoles but evidence no important changes in the structure of their walls. The chloroplasts are more widely distributed than in the normal cells but are not distorted. As in all other intumescences studied by the writer, the strongest growth lies near the veins. Often two or more small veins are involved.

Periderm development occurs in these outgrowths in the outer layers of cells. A beginning of this is shown in fig. 2, B. When periderm formation has been completed the leaf is again sealed against water loss and invasion of parasitic organisms, and the leaf completes its normal length of life without further effect of the abnormalities.

Mitchella repens. In a previous paper (4), an account was given of some experiments on leaves of *M. repens* in an attempt to determine the source of the water supply of the epidermis. In some of these experiments the lower epidermis was separated from the spongy mesophyll, and in some others the epidermis was removed from the lower surface of the leaf, which was kept afterward in a moist chamber to prevent death of the cells by desiccation. Under these circumstances the leaves threw out masses of cells from the spongy mesophyll, which had thus been relieved of pressure. All appearances would indicate that these growths were intumescences. Sections were made of representative specimens the study of which showed that masses of cells had been formed by division of the cells of the mesophyll nearest the lower epidermis. Some swelling of the cells had occurred but not enough to account for the rise of intumescences. Numerous divisions had taken place and the number of cells in an intumescence was greatly increased over that found in a section of the normal leaf. Periderm formation was found neither in the growths under the lifted epidermis nor in the leaves from which the lower epidermis had been removed. Both these situations would offer rather unsatisfactory conditions for the growth of periderm if Massart's (6) idea that cicatrization does not occur in wounds that are kept in a moist atmosphere is correct. Rodger (7), however, has shown that too much emphasis must not be put on this contention, for peri-

derm may be formed in parts of plants submerged from the time of wounding. It is still possible that the presence of a very moist atmosphere may inhibit periderm formation, where the actual water as a liquid does not.

Thurberia thespesioides. A few plants of *T. thespesioides* have been kept in the greenhouses at the Botanical Gardens for several years. For 3 successive years it has been observed that the leaves were covered with small intumescences. On some leaves little surface has been left free and normal, so that one unfamiliar with the plant might almost suppose the leaves were naturally rugose, instead of smooth, as they really are. A few leaflets could always be found that showed the normal appearance. Thus far, nothing is known of the cause of the growths on this plant.

Examination of sections of the leaves shows that the abnormalities form on either surface, though they are most numerous on the upper side. Those that appear on the upper side involve only the single layer of palisade cells and do not extend into the spongy mesophyll. The palisade layer is not affected by the outgrowths on the lower surface, which are confined to the spongy layers. The epidermal cells may or may not increase in size, but they never divide. The epidermis may be stretched to allow room for the enlarged cells inside it, but it is not often torn open, as it is in many intumescences on other plants. The relation of veins to intumescences has been noted before in this paper and also in a previous paper by the writer (5). In this plant, too, the growths are best developed near the veins.

The intumescences of Thurberia are due largely to the elongation of the cells, although some cell divisions take place. In some parts of the leaves one finds cells that have increased in width as well as in length, but by far the greater number of elongated cells show little or no widening, and, as a result, they come finally to have a most abnormal shape. (Figs. 1, D, and 2, A.) The chloroplasts of the cells are not injured and the nuclei appear normal.

Periderm seems to form in those cases where the epidermis is split open, but, as has been said, most of the intumescences never rupture the epidermis. Hence, the outgrowths cause no particular harm to the leaves on which they develop, although they remain until the leaves fall.

DISCUSSION

The intumescences described on the foregoing pages are all, with the exception of those on Thurberia, of a type that may be called "mixed," since they combine the characteristics of hypertrophies and of hyperplasias; that is, they are formed by the swelling and division of the mesophyll cells. In Thurberia the outgrowths may show a few cell divisions, but they are produced mostly by the swelling of the cells of the regular

layers of the mesophyll. Both of these types are known from the descriptions of other authors.

Küster (2) would probably not consider the abnormalities on *Hieracium venosum* and on *Mitchella repens* intumescences at all but would refer them to the category of wound reactions. It does not seem that the distinction should be pushed too far, because, after all, such growths as Küster considers true intumescences, those he has described on *Populus tremula* L., for instance, while they are not caused by wounding, probably are due to abnormal environmental conditions that may not be different in their final effect. To the writer it seems that the form of growth is a better criterion for the decision as to what name shall be applied to it than is the cause of its inception.

The development of anthocyanin, seen in the intumescences on *Eucalyptus coccifera,* has not been observed by the writer in any other plant, nor has mention been made of it by any other author so far as is known to him. Such a production of pigment is a very common occurrence in galls and also in the outgrowths of epidermal cells caused by attacks of species of Eriophyes (3). From the similarity of such growths to intumescences, it is rather surprising that the latter do not develop anthocyanin more frequently.

The development of periderm in the intumescences on *Eucalyptus coccifera* is more extensive than that observed by the writer in any other such growth or seen in the illustrations of any other intumescence on a leaf. Presumably the periderm in intumescences is dependent on the facility of periderm formation possessed by the leaf in general and shown in any type of wound on that leaf. Wylie (11) and Rodger (7) have shown that plants possess the power of periderm formation in varying degrees, and one might predict the different manifestations of this power found in different intumescences. The age of a given intumescence also has much to do with periderm formation, and it is likely that some intumescences have not been observed in sufficiently mature stages to reveal its presence.

SUMMARY

Intumescences are described on leaves of *Eucalyptus cornuta, E. coccifera, Hieracium venosum, Mitchella repens,* and *Thurberia thespesioides.*

Extensive cell division occurs in the formation of the outgrowths in the first 4 species named above, but the outgrowths on Thurberia show rather few cell divisions.

The outgrowths show extensive swelling of cells in all the species concerned, with large increases in the size of the vacuoles.

Periderm is formed in all the outgrowths except those on *M. repens*. In *E. coccifera* it is especially well developed.

Anthocyanin is formed in the intumescences on *E. coccifera*, which causes them to appear as red spots on the leaves.

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RESISTANCE OF SOME OF THE CULTIVATED SPECIES OF ALLIUM TO PINK ROOT (PHOMA TERRESTRIS)

D. R. PORTER AND H. A. JONES

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INTRODUCTION

Pink root, recently shown by Hansen (1, 2, 3) to be caused by *Phoma terrestris* Hansen, is a major disease of the onion in California. It is often found in fields growing onions for the first time, but, with continued culture in infested soil, losses ranging from 50 to 96 per cent have been reported. As the causal organism is a soil-inhabiting parasite, the most logical means of control seems to be the development of resistant strains. With this need in mind, we are now seeking resistant species of Allium or resistant varieties of *A. cepa* for hybridizing with those susceptible varieties that are important commercially, and we are selecting for resistant individuals within the susceptible varieties now extensively used in California. The marked resistance manifested by several species of Allium justifies the publication of our results to date.

HISTORICAL

Published data on resistance to onion pink root are somewhat complicated because several distinct organisms are reported as causing the disease. Taubenhaus (6, 7, 8) attributed pink root to an organism that he named *Fusarium malli* Taub., and in all his work on resistance of varieties and species he assumed pink root to be a fusarial disease. Sideris (4, 5) reported several species of Fusarium to be capable of inducing the disease. Hansen (3), however, found that onions did not become infected when inoculated with *F. malli* and stated in his paper that L. F. Alexander, of the University of Wisconsin, had obtained similar results.

Taubenhaus (8) planted the onion varieties Yellow Dutch, Yellow Danvers, Australian Brown, Large Red Globe, Large Red Wethersfield, Large White Globe, White Portugal, and Extra Early Red on infested soil and found all highly susceptible except the last mentioned variety. He thought that Extra Early Red was perhaps, in reality, Red Bermuda, which he stated was fairly resistant, as were other types of Bermuda onions, extensively grown in Texas. He further reported that the "multiplier" varieties of onions, as well as shallot and the Mexican and Italian garlic varieties, were all highly susceptible. No infection was noted on Narcissus, tulip, Funkia, Iris, Freesia, or Lilium when grown in infested soil. Hansen (3) isolated Phoma from cowpeas, *Vigna sinensis;* Lima beans, *Phaseolus limensis;* and potatoes, *Solanum tuberosum.*

We consider that pink root is caused by the species of Phoma named by Hansen, for the disease had developed in epiphytotic form when a culture of *P. terrestris* supplied us by Hansen was added to steamed soil in which onions (from disinfected seed) were growing.

METHODS AND MATERIAL

At University Farm, Davis, pink root has become particularly severe in a small plot that has been continuously planted to onions since 1925. All our tests, except when otherwise specified, have been conducted either in this infested plot or with the same soil in the greenhouse or cold frame. We know this soil to be uniformly infested because, under favorable environmental conditions, approximately 100 per cent infection develops on such varieties as Australian Brown and Yellow Globe Danvers. The methods of irrigation and cultivation are, furthermore, conducive to uniform distribution of the pathogen in the soil. Unless otherwise specified, the trials have been replicated so as to correct for possible heterogeneity of the soil. The uniformity of infection in the individual replications of the same variety gives further evidence of uniform infestation.

The species of the genus Allium that we have tested are indicated below. The commercial varieties Yellow Globe Danvers and Sweet Spanish, now known to be, respectively, highly susceptible and slightly resistant, have been used as checks in measuring the resistance among the various species of Allium. Garlic, *A. sativum,* is now extensively grown in California and Louisiana. Although leek, *A. porrum,* is not widely grown in the United States, some is produced in California. Shallots, *A. ascalonicum,* are a popular crop in certain districts of the South, especially in Louisiana. Chives, *A. schoenoprasum,* are raised only on a small scale in America. A type of *A. fistulosum,* called Nebuka by the Japanese and grown by them as a green onion, makes a rapid growth in early spring, forms no bulb, and may possibly replace shallots in some districts (Fig. 1).

Relative resistance to pink root has been measured in one of several ways. When plants were grown in infested soil in the greenhouse or in cold frames, the root systems were examined to obtain actual counts of diseased plants. Such procedure was also possible when seed was sown thickly in the field and the seedling plants were pulled for transplanting. Obviously, when leaf symptoms or yields were being determined, root infection could not always be recorded. In the field, when the plants were grown to maturity, resistance was determined by (a) percentage of plants living on successive dates during the growing season, (b) relative vigor of living plants, and (c) average weight of the mature bulbs in bulb-forming varieties or species.

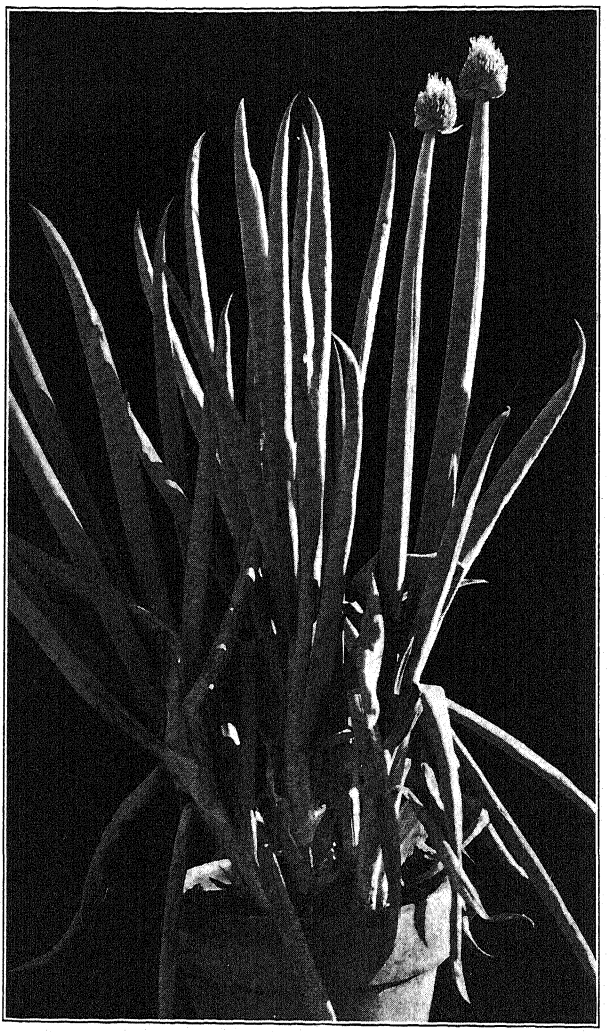


FIG. 1. Nebuka, *Allium fistulosum*, which is resistant to attack by the pink-root organism.

RESULTS

On March 15, 1928, green plants of the varieties Yellow Danvers Flat, Southport Yellow Globe, White Portugal, Australian Brown, Red Wethersfield, Southport Red Globe, Ebenezer, Yellow Globe Danvers, California Early Red, Italian Tripoli, and Valencia were planted in infested soil in the field. All the bulbs except Valencia, when dug on July 17, were found so severely infected with pink root that their average weight was 48.4 gm.

or less, while that of Valencia was 81.6 gm. Average bulb weights for the remaining varieties were as follows: Yellow Danvers Flat, 26.5 gm.; Southport Yellow Globe, 25.5; White Portugal, 25.3; Red Wethersfield, 45.8; Southport Red Globe, 35.9; Ebenezer, 36.3; Yellow Globe Danvers, 39.5; California Early Red, 48.4; and Italian Tripoli, 37.1 gm. Relative bulb size cannot always be used as an index of resistance, because of differences in date of maturity and inherent size-determining factors. Later work, nevertheless, has shown that the onions of the Sweet Spanish type consistently mature marketable bulbs in heavily infested soil.

In September, 1929, green plants of Nebuka were set in infested soil in the field. On December 31, 1929, dry sets of garlic, seed of Giant Musselberg leek, Yellow Globe Danvers, and Sweet Spanish onions were similarly planted. Except the 2 latter varieties, the growing plants were not moved until October, 1930, when all were examined for evidence of pink root. As seed of Yellow Globe Danvers and Sweet Spanish had been planted thickly, the seedlings had to be transplanted in May, 1930. At that time, infection of these 2 varieties was 98.2 and 85.1 per cent, respectively. Although 85.1 per cent of the Sweet Spanish plants were infected, many manifested only a mild attack, with a small lesion on 1 or 2 roots. Infection was more complete and severe on Yellow Globe Danvers, for, out of 1,407 plants examined, only 121 were considered sufficiently free of infection to transplant. Sweet Spanish was more tolerant of the disease than was Yellow Globe Danvers.

The results indicated that of the 6 species tested, 4, namely, *Allium porrum, A. schoenoprasum,* the Sweet Spanish variety of *A. cepa,* and the Nebuka type of *A. fistulosum,* exhibited marked tolerance of the disease, *Allium sativum* and *A. ascalonicum* were extremely susceptible; and the Yellow Globe Danvers variety of *A. cepa,* although apparently less susceptible than *A. sativum,* proved not to be so when relative plant vigor and percentage of plants severely infected were considered. Although 50 per cent of the Yellow Globe Danvers plants were living on August 25 (as measured by green leaf tissue), many of these made relatively little growth after July 8. New roots, apparently, formed just fast enough, after the older ones became infected, to keep the plants alive but not fast enough to allow normal growth. Conversely, though 96 per cent of the Sweet Spanish plants were living on August 25, not all showed the same relative vigor. Many had considerably reduced leaf area but were much more vigorous than Yellow Globe Danvers. When the bulbs were dug in September, infection of these 2 varieties, as manifested by root symptoms, was almost 100 per cent; but the Sweet Spanish had many more living uninfected roots than did the Yellow Globe Danvers bulbs. The average bulb weight of

these 2 varieties was 76.8 and 28.4 gm., respectively. Several individual bulbs of Sweet Spanish weighed as much as 280 gm., while the largest bulb of Yellow Globe Danvers weighed only 60.

Garlic and shallot were extremely susceptible. Although no complete data were taken on bulb weight of these species, an examination of the roots on August 30 showed almost 100 per cent severe infection.

Chives and leek were markedly resistant. Pink root was perhaps not severe enough on any plants of these 2 species to affect normal growth seriously. Root lesions were usually small, and very few roots showed complete infection. Similar results were secured in the greenhouse during the winter of 1930–31, when 402 chives plants were grown adjacent to 832 Yellow Danvers Flat in infested soil. The relative pink root infection of these 2 varieties was 7.9 and 94.5 per cent, respectively. Though infection on Yellow Globe Danvers was extremely severe, only very mild infection was noted on the chives roots—probably insufficient to interfere with normal growth.

Nebuka exhibited marked resistance. None of the plants died; and the roots, when examined for infection at the end of the growing season, were found only slightly damaged. This type, which grows perennially, has been propagated continuously in infested soil since September, 1929, without the slightest sign of being damaged by the disease. A similar statement may be made for chives and leek, although they have not been propagated so long in infested soil as has Nebuka.

Our next trial was conducted with infested soil in 5-in. pots set in a coldframe. The varieties used were Southport Yellow Globe, White Portugal, Sweet Spanish, Australian Brown, Prizetaker, Southport White Globe, Red Wethersfield, Southport Red Globe, Ebenezer, Italian Red, Yellow Globe Danvers, Ailsa Craig, Extra Early Red Flat, Michigan Yellow Globe, Ohio Yellow Globe, Valencia, Early Medium Yellow, leek, and Nebuka. Seed of these varieties and dry bulbs of garlic and shallots were planted on February 19, 1930. Observations were made on plant size and vigor until May 15, when the plants were pulled, weighed, counted, and examined for infection. The results (Table 1) indicate the percentage of plants moderately and severely infected as well as the calculated average weight per 100 green plants of those infected and those free of disease. These data indicate that leek and Nebuka were extremely resistant and that, of the 17 onion varieties tested, the Sweet Spanish types (Valencia, Sweet Spanish, and Prizetaker) were less susceptible than the remaining 14 varieties. Garlic and shallots were extremely susceptible.

Further tests were conducted in the field in 1930, when Nebuka, leek, garlic, shallots, and several varieties of *Allium cepa* were compared in

TABLE 1.—*Relative susceptibility of leek, Nebuka, and 17 varieties of onions to pink root, as manifested by seedlings grown in infested soil. Plants 85 days old*

Variety	Number of plants tested	Percentage of plants infected			Calculated weight per 100 plants in gm.		
		Severely	Moderately	Total	Severely infected	Moderately infected	Disease-free
Southport Yellow Globe	252	70.0	22.0	92.0	26	83	140
White Portugal	303	10.0	74.0	84.0	10	19	25
Sweet Spanish	952	7.4	66.4	73.8	20	32	39
Australian Brown	827	46.7	49.1	95.8	15	33	52
Prizetaker	307	5.0	64.0	69.0	17	33	98
Southport White Globe	601	31.5	51.5	83.0	18	38	68
Red Wethersfield	527	7.6	75.9	83.5	17	33	76
Southport Red Globe	498	10.6	79.9	90.5	19	42	61
Ebenezer	637	11.1	71.8	82.9	14	24	43
Italian Red	333	14.0	76.0	90.0	12	31	49
Yellow Globe Danvers	623	20.4	72.5	92.9	15	31	38
Ailsa Craig	305	20.0	79.0	99.0	11	36	205
Extra Early Red Flat	247	11.0	81.0	92.0	15	29	50
Michigan Yellow Globe	380	16.0	81.0	97.0	15	27	42
Ohio Yellow Globe	683	21.9	72.6	94.5	13	31	50
Valencia	336	4.0	58.0	62.0	11	26	57
Early Medium Yellow	233	0.0	71.0	71.0	.....	35	42
Leek	81	0.0	2.0	2.0	.....	20	38
Nebuka	184	0.0	3.0	3.0	.....	.....	.....
Shallots	24	26.9	63.2	90.1	.....	.....	.....
Garlic	24	20.3	71.1	91.4	.....	.....	.....

infested soil. The onion varieties planted were Yellow Danvers Flat, Southport Yellow Globe, White Portugal, Australian Brown, Southport White Globe, Red Wethersfield, Southport Red Globe, Ebenezer, Italian Red, Yellow Globe Danvers, Early Medium Yellow, Yellow Strasburg, Ohio Yellow Globe, Valencia, Sweet Spanish, and Prizetaker. Green plants grown for several weeks in disease-free soil in a cold frame were transplanted into an infested area in the field on May 15. Observations were made regarding percentage of living plants and relative vigor during the growing season; and, as the varieties matured, bulb size and percentage of plants producing bulbs were determined. Summarized data covering this test (Table 2) show that at maturity, in infested soil, those varieties of the Sweet Spanish type produced significantly larger bulbs than did the other varieties. Evidently, furthermore, Nebuka and leek were more resistant than any varieties of *A. cepa* used. No data are given for garlic and shallots in this test, but the plants died well in advance of the normal date of maturity, the bulbs were stunted, and much of the root system was destroyed by the pink-root pathogen.

TABLE 2.—*Response of some species of Allium and of 15 varieties of A. cepa to pink root when green plants produced in disease-free soil were transplanted into infested soil in the field on May 15, 1930, at Davis, California.*

Variety	Number of plants	Percentage of plants living		Relative vigor[a]		Percentage bulbing	Average bulb weight gm.
		June 18	July 19	June 18	July 19		
Yellow Danvers Flat	75	92.0	66.6	3	2+	85	33.6
Southport Yellow Globe	79	91.1	84.8	3	3	93	30.8
White Portugal	79	96.2	89.9	3	3–	94	26.1
Australian Brown	80	88.7	62.5	2	2–	87	18.3
Southport White Globe	80	98.7	85.0	4–	3	90	39.2
Southport Red Globe	79	96.1	81.1	3	3–	94	25.2
Ebenezer	79	91.1	66.1	2	2	98	16.9
Italian Red	80	90.0	82.5	2+	2+	87	30.8
Yellow Globe Danvers	79	87.4	79.8	3–	3–	85	24.4
Early Medium Yellow	80	96.3	85.0	2+	2+	94	22.4
Yellow Strasburg	158	94.3	75.1	3–	2	82	24.4
Ohio Yellow Globe	156	95.1	74.2	2+	2	91	22.9
Sweet Spanish	237	98.9	97.1	4	4–	98	61.6
Valencia	77	98.2	96.1	4	3+	99	58.8
Prizetaker	79	97.1	96.0	4	3+	98	64.4
Nebuka	45	100.0	100.0	4	4	.....	.....
Leek	45	100.0	100.0	4	4	.....	.....

[a] Indicated progressively from 0 (plants dead) to 4 (most vigorous).

The final test was conducted in the greenhouse with Yellow Globe Danvers (susceptible) and Nebuka (resistant) in soils that varied in degree of infestation. Steamed and field-infested soils were used as checks on infested soil that had been diluted with steamed soil and placed in separate compartments of a bench in the greenhouse. The dilutions were 1:10, 1:5, and 1:1 of infested with steamed soil. Seed was planted on March 24, 1931; and final readings were made on May 26, when the plants were approximately 2 months old.

TABLE 3.—*Relative resistance to pink root of Nebuka and Yellow Globe Danvers when grown in soil that varied in degree of infestation. Data from seedling plants in the greenhouse, 1931.*

Soil infestation	Variety	Number of plants	Percentage free of pink root	Percentage of plants infected			
				Severely	Moderately	Slightly	Total
None (steamed soil)	Danvers	2,519	100.0	0.0	0.0	0.0	0.0
	Nebuka	369	100.0	0.0	0.0	0.0	0.0
1:10[a]	Danvers	2,601	42.7	1.9	21.8	33.6	57.3
	Nebuka	628	89.1	0.0	0.0	10.9	10.9
1:5	Danvers	1,647	26.3	7.9	41.1	24.7	73.7
	Nebuka	589	85.2	0.0	0.0	14.8	14.8
1:1	Danvers	2,270	20.1	22.1	33.9	23.9	79.9
	Nebuka	517	72.3	0.0	0.6	27.1	27.7
1:0 (field infested)	Danvers	1,844	9.4	25.4	34.3	30.9	90.6
	Nebuka	584	71.9	0.0	2.3	25.8	28.1

[a] One part field-infested to 10 parts steamed soil.

The results of this final test (Table 3) indicate further that Nebuka exhibited marked resistance to pink root under conditions made favorable for infection where the entire root system could be examined. Although only 71.9 per cent of the Nebuka plants were entirely disease-free when grown in field-infested soil, almost none of the plants was sufficiently infected to interfere with normal growth. One should note that the percentage of infection increased slightly with increased degree of soil infestation. None of the Nebuka plants was severely infected; 1.4 were moderately and 17.3 per cent slightly infected. When compared with Yellow Globe Danvers, furthermore, Nebuka was markedly resistant to the pink root, regardless of the degree of soil infestation.

SUMMARY

Varieties of 6 species of Allium have been tested for resistance to onion pink root, caused by *Phoma terrestris*. *Allium fistulosum* (Nebuka type), *A. porrum* (leek), [(Giant Musselberg variety)], and *A. schoenoprasum* (chives) have proved extremely resistant. *Allium sativum* (garlic), *A. ascalonicum* (shallot), and most varieties of *A. cepa* (common onion) are extremely susceptible, although the variety Sweet Spanish may be classed as moderately susceptible.

DIVISION OF TRUCK CROPS,
BRANCH OF THE COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA, DAVIS.

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TUBERCULINA MAXIMA IN WESTERN NORTH AMERICA

J. L. MIELKE[1]

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INTRODUCTION

The lilac fungus (*Tuberculina maxima* Rost.) has long been known as a parasite of white-pine blister rust (*Cronartium ribicola* Dietr.) in Europe (8, p. 160; 11) and on that Continent appears to be widely distributed on this host (1, p. 252; 5; 10; 14; 18). Although it is known upon several of our native pitch-pine blister rusts (*Cronartium commandrae* Pk. (16, 17), *C. coleosporiodes* (Diet. & Holw.) Arth., *C. cerebrum* Arth. & Kern (17), *C. harknessii* (Moore) Meinecke, *C. stalactiforme* Arth. & Kern (16), and *C. quercus* Schrt. (9, p. 12), it has not been reported on this Continent upon the white-pine blister rust (10), or upon *C. comptoniae* Arth., the sweet-fern blister rust of pitch pines. This paper reports the discovery, distribution, and observed behavior of *T. maxima* upon these two rusts in western North America.

DISCOVERY, HOSTS, AND DISTRIBUTION

Tuberculina maxima[2] as a parasite of *Cronartium ribicola* was first observed by J. S. Boyce, of the Division of Forest Pathology, on cankers on western white pine (*Pinus monticola* D. Don). It was found on August 18, 1926, at Daisy Lake, British Columbia. At this same place *T. maxima* was found on *C. comptoniae* for the first time on April 25, 1931, when the writer collected it on cankers caused by this rust on lodgepole pine (*P. contorta* Loud.). This appears to be the first record of *T. maxima* on this host in North America.

Following the discovery made by Boyce at Daisy Lake, B. C., in 1926, additional records of the occurrence of *Tuberculina maxima* on *Cronartium ribicola* were made during the fall of that same year. The writer found it at another locality at Daisy Lake on October 16 and also at Brackendale, B. C., on October 18. H. G. Lachmund, of the Division of Forest Pathology, noted it at Owl Creek, B. C., on October 17. Since these observations,

[1] The writer wishes to express his gratitude to E. P. Meinecke, Principal Pathologist, Division of Forest Pathology, U. S. Bureau of Plant Industry, for his kind assistance in supplying some of the literature references and for translations of some of the foreign papers.

[2] This fungus was determined as *Tuberculina maxima* Rost. by Boyce, verified by J. R. Weir, and critically compared with European material by C. von Tubeuf of Germany, who confirmed the previous determinations.

the parasite has been noted in only one additional locality in the coastal section—near Little Blackwater Lake, a few miles west of D'Arcy, B. C., where it was found by the writer on June 19, 1930.

The range of *Tuberculina maxima* on *Cronartium ribicola* as now known is confined to a narrow strip of country bordering the line of the Pacific Great Eastern Railway, beginning at Brackendale, B. C., about 30 miles north of Vancouver, and extending northward for about 60 miles to the vicinity of D'Arcy, B. C. Daisy Lake is about 13 miles and Owl Creek about 45 miles north of Brackendale.[3] On *C. comptoniae* the parasite has been found only at Daisy Lake.

This comparatively limited distribution of *Tuberculina maxima* does not result from limited occurrence of these two western hosts. *Cronartium ribicola* was known to be widely distributed on *Pinus monticola* in both the coastal and interior white-pine belts of British Columbia in 1923 (6). The present known range of this rust on western white pine is from near the northern tip of Vancouver Island in British Columbia to the central Cascade Mountain region of Oregon and from the Pacific Coast eastward nearly to Montana. The main range of *C. comptoniae* on *P. contorta* in western North America as outlined by Lachmund (3) is from Prince Rupert, B. C., southward through the coastal region to the mouth of the Columbia River in Washington. The coastal portion of the range of *C. ribicola* on *P. monticola* is, therefore, fairly comparable to the main range of *C. comptoniae* on *P. contorta*. In this part of the country much intensive research has been conducted on *C. ribicola* during the past 10 years. Considering, therefore, the few localities at which *T. maxima* has been noted in this connection, it seems apparent that the occurrence of the parasite is decidedly limited relative to the range of these two rusts.

PARASITE CONSIDERED INDIGENOUS TO THE REGION

If the lilac fungus were introduced with the white-pine blister rust into this region from Europe, one would expect to find it associated at or near the point where the latter was introduced into western North America at Point Grey, near Vancouver, B. C., in 1910 (2). There is, however, no evidence to this effect. Several blister-rust investigators are known by the

[3] Since this paper went to press *Tuberculina maxima* has been found during the summer on *Cronartium ribicola* in the interior section of British Columbia and southward in the United States. The stations are as follow: Revelstoke, B. C., by E. L. Joy, U. S. D. A., Div. Blister Rust Control, Spokane, Wash., Arrow Park and near Rosebery, B. C., by members of the Div. Forest Pathology, U. S. D. A., Portland, Oregon, and Newman Lake, Wash., by R. K. Pierson, U. S. D. A., Div. Blister Rust Control, Spokane, Wash. *T. maxima* was noted to be rare at all these localities except Arrow Park where it was frequently encountered. All localities are old blister-rust infection areas, the majority originating in 1923 and one (Revelstoke) as early as 1917 or 1918.

writer to have examined the infected pines at this original introduction point, and none of them reported the presence of *Tuberculina maxima.* Furthermore, it has never been noted on infected pines anywhere else in the vicinity of Vancouver. On the other hand, there are good indications that the lilac fungus is indigenous to North America. As has been mentioned, this parasite has been found abundant on *Cronartium comptoniae* on *Pinus contorta* near Daisy Lake, B. C., and is known to occur in the United States on several other Cronartium species.

BEHAVIOR OF TUBERCULINA MAXIMA IN BRITISH COLUMBIA

The parasite attacks both pycnia and aecia of the white-pine blister rust. In British Columbia it has been observed that production of the aeciospores is greatly reduced when the aecia are attacked and that death of the bark over the cankered area often occurs in a few years. Rust-infected bark of stem cankers on trees 3 to 8 in. in diameter when parasitized may become noticeably constricted prior to death of affected parts. In Germany, Tubeuf (15) found by inoculations that *Tuberculina maxima* suppressed formation of aecia and repeatedly led to a dying of the bark. The writer has observed that on aecial fructifications the parasite commonly develops a tuberculate formation.

In British Columbia pycnia may be attacked throughout the entire seasonal period of their production, which is generally from June to September, inclusive. A canker parasitized during the first part of the season may produce pycnia all summer, but the new pycnia are generally attacked soon after their appearance. This attack on the pycnia no doubt reduces the volume of pycnospore production. Spaulding (10) observed that in Europe the pycnospores are not produced in great abundance, partly because of the effect of *Tuberculina maxima,* which reduces locally their production in diseased bark.

Sporulation of the parasite on the pycnial stage of the rust is very abundant under British Columbia conditions, particularly in summer during the main period of pycnial production. At this time, cankers have been observed with their pycnial area completely covered by a thick dust-like layer of the lilac-color spores—a phenomenon that has not been noted in like degree during the period of aeciospore production. A pycnial zone attacked one season usually fails to produce aecia the following year.

All indications are that the pycnial fluid is the principal substratum for the growth and development of the lilac fungus. Field observations to this effect are borne out by the results of a study conducted at the suggestion of the writer by Professor H. P. Barss, Oregon State College, Corvallis, Oregon. He found that an aqueous solution of the pycnial fluid was an excellent medium for the germination of *Tuberculina maxima* spores.

This apparent preference of the parasite for growth on pycnia indicates that its effect on aecial production is indirect. Evidence substantiating this has been obtained. During the spring of the past year, at the time aecial sporulation was in progress, specimens of *Tuberculina maxima* were collected from a parasitized stem canker by shaving off portions of the infected bark with a knife. The cuts in the bark were not quite deep enough to remove the cambial layer. This same canker was examined about a month later, and *T. maxima* was then found to be sporulating profusely on portions of the cut surfaces. This seems to indicate that the parasite is not entirely superficial upon pycnia and aecia but that its mycelium invades the bark well beneath these fructifications on the surface. Otherwise, it would not have been able to sporulate so promptly when the outer bark was removed. If this is the case, it may explain why a pycnial zone attacked one season usually fails to produce aecia the following year. It may also explain why the lilac fungus appears to be a factor in hastening the death of cankered bark, which usually remains alive and sporulating for 3 or 4 years in any particular canker zone after the first appearance of aecia. As has been mentioned, Tubeuf (15) found that cankered bark dies after invasion by *T. maxima*, and this has been a common observation of the writer.

Tuberculina maxima has not been reported in Europe on the uredinia and telia of the white-pine blister rust on species of Ribes,[4] nor has it ever been observed on these stages of the rust in North America.

Observations on the behavior of the lilac fungus on the sweetfern blister rust were confined to the Daisy Lake area and were all made during the season of 1931. The data secured indicate that the action of the parasite on this rust is similar to that on the white-pine blister rust. It attacks both pycnia and aecia but sporulates in greatest abundance on the pycnia, which are produced during summer. The parasite does not appear to be generally destructive to *Cronartium comptoniae*.

IMPORTANCE IN THE CONTROL OF WHITE-PINE BLISTER RUST

At present there is no evidence that under British Columbia conditions the lilac fungus can effectively suppress the white-pine blister rust. Comparing the known extensive range of *Cronartium ribicola* with the limited area in which *Tuberculina maxima* occurs, it is apparent that the latter lacks aggressiveness and is very tardy in the invasion of this host. Examples of this have been observed on several occasions. At three localities where the parasite was noted on a few cankers in 1926, no trace of it was found after a diligent search in 1931. During the intervening years it was

[4] The genus Ribes is used in this paper to include both currants and gooseberries.

rarely in evidence at these same localities. At Owl Creek, however, where experiments in the control of blister rust by the lilac fungus. The mode the parasite was noted as common in 1926 and very common in the following years, it may still be frequently found, yet its presence has not resulted in measurable reduction of blister-rust attack. The first damage by blister rust to western white pines at this place was also noted in 1926, when a few flags (dead branches) could be seen on these trees in an otherwise healthy-appearing stand of white pine and Douglas fir reproduction in approximately equal representation. Today the stand is almost pure Douglas fir, practically all the pines having been killed by the rust in spite of the abundance of the parasite. In this case, even with *T. maxima* sporulating abundantly each year, not all of the aecia were parasitized and aeciospore production was sufficient to cause abundant annual infection of Ribes with a resultant continued infection and killing of the pines. The other localities where the lilac fungus has been found are some of the oldest and most severe pine-infection centers in this region, and yet in these places the parasite has barely managed to perpetuate itself.

It is not uncommon to observe one tree of western white pine with numerous blister-rust infections, many of which are invaded by the parasite, while a few feet distant may be another similarly infected tree of about equal size on which no trace of the lilac fungus can be found. Also, on numerous occasions heavily infected pines have been observed with only one or two parasitized cankers.

This sporadic occurrence and behavior of *Tuberculina maxima* may indicate lack of virulence that, as compared with its more aggressive behavior as reported in Europe, suggests the possibility of different strains of the organism. If this be the case, it still remains to be seen just how readily the European fungus would attack blister rust in this country. Experiments and investigations in Europe (4, 10, 12, 13, 14, 15), however, have apparently not resulted in successful control of the rust with the aid of the parasite, for, in spite of its presence, the destruction of white pines by the rust in that country has continued. Perhaps it might be suggested that the blister rust in western North America is caused by a strain of *Cronartium ribicola* that is less susceptible to *T. maxima* than the common European strain. No positive indication, however, of the existence of physiological strains of blister rust has ever been found (10); hence, it is unlikely that this is a factor in the behavior of the lilac fungus. It is far more probable that its erratic behavior is caused by environmental conditions unfavorable to its propagation and spread.

Tuberculina maxima may offer some possibilities with respect to local control of the rust. Rohmeder (7) states that good results have been ob-

tained in several Swabian forest areas in controlling *Cronartium ribicola* by its natural enemy, *T. maxima*. Tubeuf (14) has reported successful of dissemination employed by the latter consisted of collecting the lilac-color spore dust of the parasite in a paper bag and transferring it to the aecia with the tip of a penknife. This procedure would, of course, be impracticable in extensive control operations such as are employed in this country.

Tubeuf (14) emphasizes that never are all white pines killed by the rust nor are all rust foci destroyed by *Tuberculina maxima* and that one may speak only of reduction, not of eradication. The writer knows of no instance of successful biological control of an active fungous parasite of plants. It appears, therefore, that little hope may be held for *T. maxima* as an important factor in the general control of *Cronartium ribicola* in this country.

SUMMARY

The lilac fungus (*Tuberculina maxima*), a parasite of the pycnia and aecia of several pine rusts, is reported for the first time in North America on *Cronartium ribicola* and on *C. comptoniae*.

The known distribution of the parasite on *C. ribicola* is limited to two small areas in British Columbia—one in the coastal and the other in the interior region—and to one locality in northeastern Washington. On *C. comptoniae* the parasite has been found only at Daisy Lake, B. C., in the coastal region.

There is no evidence that *T. maxima* was introduced into this country with the introduction of *C. ribicola* to western North America. There is evidence, however, that it is indigenous, probably on one or all of its native pitch-pine blister-rust hosts.

The parasite has never become generally destructive to either *C. ribicola* or *C. comptoniae*. It is not considered an important factor in reducing aecial sporulation or in controlling *C. ribicola*.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
PORTLAND, OREGON.

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ASPERGILLUS SCLEROTIORUM, N. SP., AND ITS RELATION TO DECAY OF APPLES[1]

G. A. HUBER

(Accepted for publication May 18, 1932)

From 1926 to 1929 11 forms of Aspergillus were isolated by the writer from the surface of normal apples (5). Seven of these were found capable of causing decay on apples in common storage; none, however, caused decay in cold storage (0°–2° C.). Several species of Aspergillus have been isolated by other investigators (2, 3, 4, 7) from common storage and market apples, but none has been reported to cause decay in cold storage. Brooks and Cooley (1) found that apples inoculated with *Aspergillus niger* (Cram.) v. Tiegh., kept in the laboratory for 1 week after inoculation and then placed in cold storage, decayed. Apples, similarly treated and placed in cold storage immediately after inoculation, did not decay. In explanation of these results, they state that "all or nearly all of the rot was produced before the fruit was cooled."

Since 1929 3 additional forms of Aspergillus have been isolated by the writer from the surface of normal apples. Only one of these forms proved pathogenic on apples, causing decay at both common and cold-storage temperatures. When sound, ripe Jonathan apples were inoculated with mycelium and spores of this species and immediately placed in cold storage, a distinct decay was produced. This organism is presented here as a new species belonging to the *A. ochraceus* group, *sulphureus* series (9). The following description was made from cultures growing on Czapek's solution agar, as used by Thom and Church (9):

Aspergillus sclerotiorum, n. sp.

Colonies (Fig. 1, A) white at first, becoming sulphur yellow (Ridgway, Pl. V, 25) (8) with the maturing of the conidial heads and thickly seeded with light sclerotia; growth slow, reaching a diameter of 35 mm. in 10 days at 25° C.; reverse cream color. Conidial heads (Fig. 1, C, D) varying in the same colony from hemispherical to columnar, more or less loose, commonly splitting into 2 or more divergent columns, varying in size up to 140 μ wide with the hemispherical heads and up to 250 μ long by 140 μ wide with the columnar heads. Stalks light yellow, pitted, up to 1,200 μ long by 6 μ to 12 wide, slightly increasing in width toward the conidial heads. Vesicles globose to flask-shape, up to 40 μ in diameter. Sterig-

[1] Published as Scientific Paper No. 202, College of Agriculture and Experiment Station, State College of Washington.

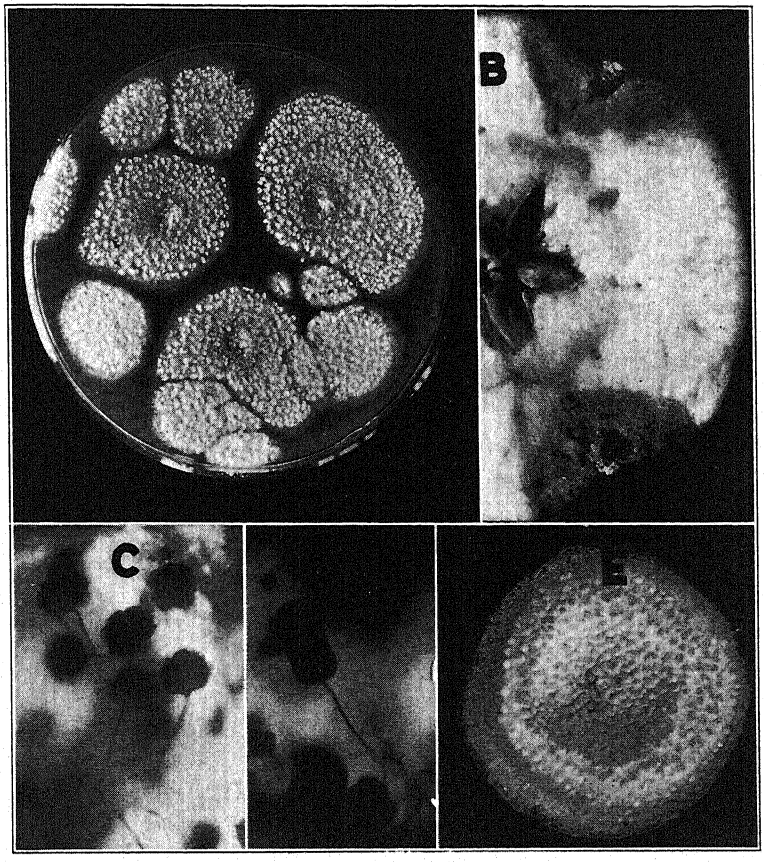


FIG. 1. *Aspergillus sclerotiorum.* A. Plate culture on Czapek's solution agar incubated at 25° C. for 12 days. B. Cross section of Jonathan apple, showing type of decay. C and D. Photomicrographs, showing hemispherical and columnar conidial heads, respectively. × 50. E. Carrot slice, showing abundant sclerotial development.

mata in 2 series, primary and secondary, approximately equal in length, up to 8.5 μ. Spores globose, smooth, 2 μ to 3 μ in diameter; walls slightly tinged with yellow. Perithecia not found. Sclerotia abundant, first appearing on cultures 3 days old, globose to subglobose, white at first, soon becoming light cream and then flesh pink (Ridgway, Pl. XIII, 5) (8), up to 1.5 mm. in diameter.

On sterile carrot slices,[2] mycelium white at first, turning pink with the beginning of sclerotial development. Conidial production sparse. Sclerotial development very abundant (Fig. 1, E).

[2] Sliced carrots placed in Petri dishes on wet filter paper and sterilized in an autoclave for 20 minutes at 15 pounds' pressure.

When inoculated (6) into sound, ripe Jonathan apples, the fungus produced lesions 32–36 mm. in diameter in 28 days at 22–25° C. In 42 days the lesions had attained diameters of 42–46 mm. Held in storage at 6–8° C., the fungus produced lesions 16–23 mm. in diameter in 42 days. At the end of 90 days the lesions had attained diameters of 28–38 mm. In cold storage (0–2° C.), it produced lesions 10–14 mm. in diameter in 120 days. The surfaces of the lesions were brown with a yellowish green tinge, slightly sunken, and tending toward concentric rings, which were very pronounced on the colored cheeks of the apples. The decayed tissue was dark tan, dry, and more or less spongy. The lesions generally had the form of flattened cones (Fig. 1, B), the diameter of the lesions exceeding the depth to which the decay penetrated.

SUMMARY

A new species of Aspergillus is described as belonging to the *A. ochraceus* group, *sulphureus* series. This organism, for which the name *A. sclerotiorum* is suggested, was found to be pathogenic on apples, causing decay both at ordinary and cold-storage temperatures. When inoculated into sound, ripe Jonathan apples it produced lesions 42–46 mm. in diameter in 42 days at 22–25° C. It produced lesions 28–38 mm. in diameter in 90 days at 6–8° C. and lesions 10–14 mm. in diameter in 120 days at 0–2° C.

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PHYTOPATHOLOGICAL NOTES

Bacterium cucurbitae on cucumber.—In describing the bacterial leaf spot of squash (*Bacterium cucurbitae*), the writer[1] made the statement that this disease had not been found on cucumbers and that inoculations on this plant produced pin-point infections that did not develop further. Prasad,[2] however, in 1931, reported finding in India a leaf spot of cucumber caused by an organism, which he proved to be pathogenic to cucumber and which he states is identical with *Bact. cucurbitae* in cultures. The spots are described as thin, brown, and water-soaked, and made conspicuous by a bright yellow halo.

The disease has now been found on cucumber in the United States, though doing only minor damage. (Fig. 1, A.) Early in September, 1931, O. C. Boyd[3] collected specimens in Bristol County, Massachusetts, which he forwarded to the writer with the following comment: "Earlier in the season I saw no typical angular leaf spot on cucumber in that county. This spot on rather old vines just about through bearing, does not appear much like angular spot, yet bacteria flow out profusely from sections." Isolations from five widely separated spots on these leaves produced only *Bacterium cucurbitae.* This organism was also isolated from Hubbard squash leaves from the same county; concerning its prevalence on squash, Boyd says:[3] "The latter leaf disease was to be found in every field I visited toward the end of the season and very severe in certain fields." It seems probable that here the disease spread from squash to cucumber. Inoculations have been produced on cucumber and squash with the isolations from the cucumber leaves, reisolations made, and infection again produced on cucumber. (Fig. 1, B.)

Lesions on cucumber may be round or somewhat angular. The thin, papery brown center has a reddish brown margin and may or may not be surrounded by a yellow halo. There is less tendency than in angular spot for the centers to tear out. The diseases are sufficiently similar, however, to make field identification difficult. MARY K. BRYAN, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

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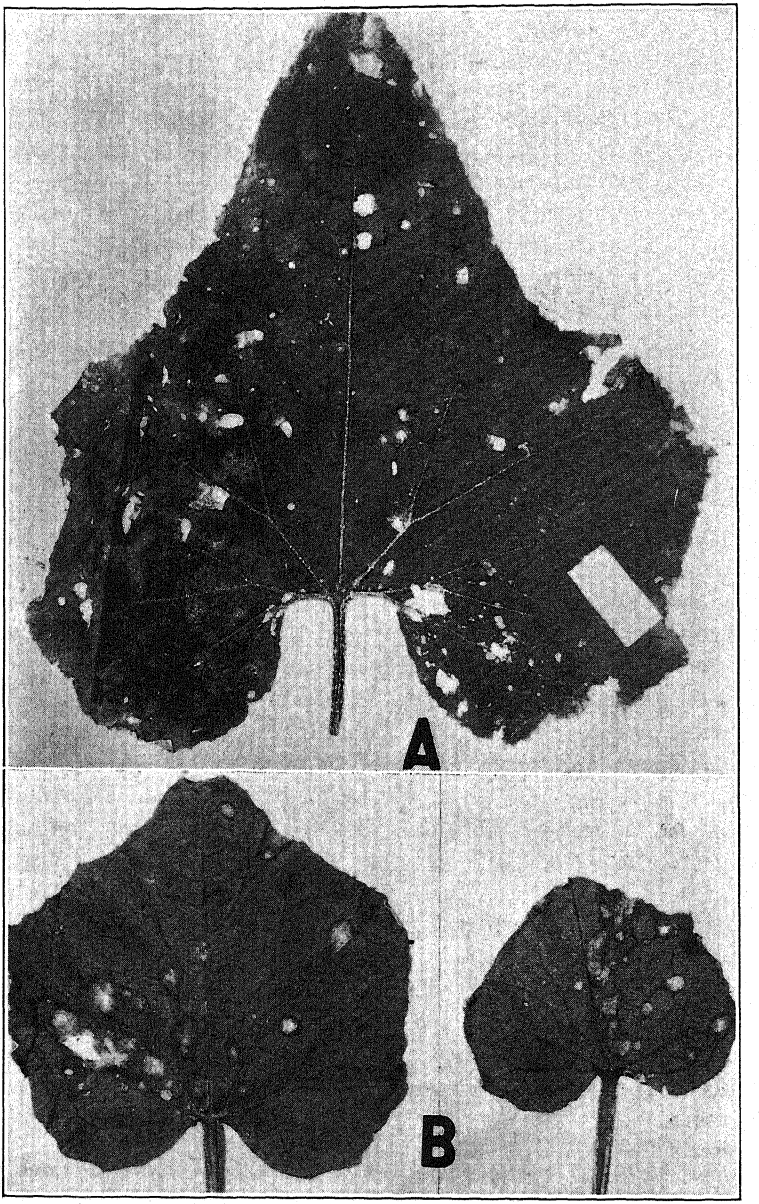


FIG. 1. A. Cucumber leaf collected in Massachusetts from which *Bacterium cucurbitae* was isolated. B. Cucumber leaves from a young plant sprayed with the isolation from leaf shown in A.

Cucumber Mosaic on Tobacco in Wisconsin.—Natural field infection of tobacco with the cucumber-mosaic virus has previously been known to occur in small percentages in different States. In 1932, field observations of mosaic symptoms on tobacco in Wisconsin suggested the widespread occurrence of the cucumber-mosaic virus on this host. In order to verify this observation, samples from 60 tobacco plants, representing 25 fields in various parts of the State, were selected and tested by inoculation to young greenhouse plants. Twenty-six plants yielded typical cucumber-mosaic virus; 21 yielded ordinary tobacco-mosaic virus; and in 13 cases the specific virus was apparently neither of the above but was not definitely identified. Plants that yielded ordinary tobacco-mosaic virus may in some instances have contained cucumber-mosaic virus also, since no separation of possible combinations was attempted. In one station field at Madison, about 30 per cent of the plants were affected with cucumber mosaic and a high percentage with ordinary tobacco mosaic. Judging from the symptoms manifested, cucumber mosaic was present in still higher percentages in parts of other fields in the State. More limited observations and inoculations indicated a similar situation with respect to mosaic on tomato. It was evident that cucumber mosaic was occurring in epidemic form on tobacco in 1932 in Wisconsin. On the basis of field observations in past years, it is believed that the occurrence of this disease on tobacco in epidemic form is unusual. The mode of transmission in this particular epidemic was not determined. Should cucumber mosaic occur regularly on tobacco in association with ordinary tobacco mosaic, control measures will naturally be complicated.—JAMES JOHNSON, Wisconsin Agricultural Experiment Station and U. S. Department of Agriculture.

Insects and Diseases of Ornamental Trees and Shrubs. By E. P. Felt and W. H. Rankin. xix + 507 pp., 243 figs. The Macmillan Co., New York. 1932.

In this volume the authors have presented a combined revision of their former books, "Manual of Tree and Shrub Insects", by E. P. Felt, and "Manual of Tree Diseases", by W. H. Rankin. Some portions have been rewritten with additions of more up-to-date information, but much of the subject matter is simply a reorganization of the previous books. The volume is one of "The Rural Science Series" and is therefore of a semi-popular nature. Since plant pathologists will be interested particularly in the presentation of the diseases, this review will be concerned chiefly with that phase of the volume.

The book is divided into 2 parts, the first designated as "General: insects, fungi, injuries and treatments". There are 4 chapters in this section, covering 131 pages. The first chapter, of some 38 pages, deals with "Insects and their depredations". The second chapter, conforming with this first heading, is entitled "Fungi and their depredations". The 60 pages of the chapter are devoted to brief discussions of the following subjects—How the organism causes diseases; Diseases common to all trees—including leaf spots, powdery mildews, sooty molds, leaf cast of conifers, slime flux, lichen injury, mistletoe parasites, and the control measures in each case; Wood decay and root diseases, including heartwood decays, wound decay, basal decay and root diseases; Wounds and cavities in deciduous trees with treatments. Although much of this material was contained in the earlier "Manual of Tree Diseases", the concise description of the manner in which infection takes place and the relation of factors of environment will be a welcome addition for the general reader. The last subject in the chapter—wounds and cavities with treatments—is new material and takes the place of the chapter on "Tree Surgery" in the earlier "Manual". However, the author does not recommend that the tree owner undertake the treatment of wounds but emphasizes his belief that "Such work, as well as the more intricate methods of treating cavities, must be done by those with experience, the proper tools and accredited training in definite methods." He states briefly the principles underlying wound treatment and outlines the various processes of cavity treatment used in the past. All discussion of specific materials and equipment for cavity filling is omitted, and the reader is not referred elsewhere for such information.

Chapter III, entitled "Insecticides and Fungicides", describes Bordeaux, lime-sulphur and sulphur dust or spray. In the 3 paragraphs devoted to "Spraying Apparatus" the qualifications of a satisfactory apparatus are given, but undue emphasis has been placed on the difficulties in-

volved in the process of spraying, so that tree owners or gardeners would hesitate to undertake it themselves. In fact, the authors state that it requires expert knowledge to determine the proper time for spraying and that only well-qualified men should be intrusted with such work. It is evident that the book is written from the standpoint of the commercial arborist, who is not interested in explaining to amateur gardeners how to carry out the control measures themselves.

"Injuries other than those caused by organisms" are described in the 20 pages of Chapter IV. The first three headings—Root injuries, Body and branch injuries, and Leaf injuries—cover general discussions of the relation of environmental factors to the health of the various parts of the tree. These are followed by the descriptions of specific injuries in much the same manner as in the "Manual" with headings:—Injuries due to water relations; Freezing injuries; Gas and smoke injuries; and Electrical injuries.

Part II is devoted to an account of specific insects and diseases that attack ornamental trees and shrubs. The arrangement is according to the alphabetical listing of about 70 generic hosts, each genus being designated by its common English name. In a few cases, the separation of species of a generic host is made when the common names of the species differ, for example, azalea and rhododendron, buckeye and horse chestnut, cherry and plum. About 40 of the hosts are additions to the list in the "Manual" and include ornamental fruit trees and shrubs. Insect pests are described for all of these new hosts, but diseases are mentioned for only half of them. For many hosts the discussion of diseases and insects conforms to a definite arrangement with paragraph headings:—Insects attacking leaves; Leaf diseases; Insects attacking twigs and bark; Diseases of twigs and bark; Insects of trunk and wood; Diseases of trunk, wood and roots. Unfortunately, this arrangement cannot be consistently maintained, since for some of the hosts very few diseases have ever been reported. In a few cases, however, there is no apparent reason for the digression from the general scheme. Usually, each disease is described in a concise paragraph, including distribution, causal fungus, symptoms, and control. In most cases, the first word or words of a paragraph indicate the common name of the disease to be described. Here, again, there is frequently a digression from this scheme, sufficient to cause confusion in locating readily a particular disease. The less important diseases are sometimes grouped in one paragraph with a few sentences devoted to each disease. Unless one knows the name of the causal organism, it is difficult to locate the description of any particular disease. For example, 5 apple cankers are described in 1 paragraph covering 2 pages with only the fungus names in italics to distinguish them readily. A very complete index, however, tends to counteract the difficulties of the arrangement.

The book, as the authors state in the preface, "does not represent the final word," but "is a summation of available information to date." On the whole, a careful selection of the most common diseases has been made, including those that have not yet been satisfactorily studied but that have already given indication of their importance. In the cases of some of the shrubs, however, brief mention of a few other common diseases might have been included, such as the mildew of crape myrtle, leaf spots of holly and of English ivy. The omission of such mention gives an erroneous impression that no diseases have been reported on these hosts.

The most regrettable change from the earlier "Manual" is the complete elimination of a bibliography. This seems particularly unfortunate in connection with those diseases that have been recently reported or in which the causal organisms have not yet been discovered. Undoubtedly, the value of the book for readers familiar with fungi and for students of plant pathology is considerably lessened by this omission, since additional information will be desired to supplement the brief descriptions and to clarify vague statements. The book is intended primarily for those unacquainted with the technical aspects of tree diseases but interested only in finding out what is the cause of certain symptoms of disease. For this purpose the information is adequate and the book is an acceptable addition to the "Rural Science Series".—ALMA M. WATERMAN, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Brown University, Providence, R. I.

Shear, C. L., Neil E. Stevens, and Henry F. Bain. Fungous Diseases of the Cultivated Cranberry. U. S. Dept. Agr. Tech. Bul. 258: 1–56 pp. 4 pl. (2 col. pl.), 35 fig. 1931.

To consider in the category of a book a bulletin, regardless of the source, may seem somewhat out of place; yet, when that bulletin is a plant-disease monograph, it changes from a run-of-the-mill publication of scientific research and becomes a reference work. Hence, the publication by the United States Department of Agriculture of "Fungous Diseases of the Cultivated Cranberry," by C. L. Shear, Neil E. Stevens, and Henry F. Bain, is of especial moment.

About 3 years ago, it was my pleasure and privilege to chronicle the advent of Harter and Weimer's "A Monographic Study of the Diseases of Sweet Potatoes and their Control," the first of such publications to be issued by any organization. Now, it is my good fortune to call the attention of plant pathologists and mycologists to a similar monograph, similar, however, in that it is a monographic treatment of diseases. Where Harter and Weimer devoted their space principally to the several diseases as conditions that demand prophylactic and other treatment, the present authors consider diseases more in the light of their causal organisms. In fact, 39

of the few more than 50 pages of text are devoted to the taxonomy and physiology of the rot-producing fungi.

Whether the discussion is of important rot fungi, fungi causing diseases of cranberry vines, or cranberry fungi of minor importance, the general treatment, taxonomically, is the same, for an example of which the treatment of storage-rotter *Diaporthe vaccinii* may be cited: Synonymy, pycnidia, perithecia, type specimen, cultural characters on artificial media, hosts, geographical distribution, genetic relationship between Phomopsis and Diaporthe, pathological relations.

Into the physiology of the rot fungi the authors have delved at some length, taking up in order time of infection, dissemination by water, aridity relations, temperature relations, relative abundance of cranberry fungi in different regions (with considerations of the field rots, relative importance of different fungi as the cause of storage rots, and succession of fungi in storage), climates of different cranberry sections in relation to the abundance of various fungi (including a discussion of the factors bearing on sterile breakdown), and the relation between growing-season weather and keeping quality of the cranberry crop in Massachusetts.

The control of the fungous diseases of cranberries is discussed under the headings of vine diseases and cranberry fruit rots. In the former case, individual diseases—fairy ring, red gall, rose bloom, red leaf spot, black spot, hard rot (cotton ball) and tip blight—are briefly treated, as they "are field diseases and may fairly be classed as minor troubles."

With fruit rots, however, it is another story, and the authors go into some detail regarding the control of these diseases, first pointing out the differences in climate, cultural conditions, varieties, and marketing policy in the several cranberry-growing regions, which are of significance in control measures. As to actual measures for control, spraying with Bordeaux 4–4–½–50 (the ½ being commercial fish-oil soap) is but one of 6 headings under which control measures are advocated, the other 5 being bog management, harvesting methods, handling methods, storage, and marketing the crop.

Synonyms for names of host plants, a "Literature cited" of 86 titles, and an index to fungi are given.

Small in size, this cranberry-disease monograph is great in its make-up and usefulness. Any one who has had occasion to search the literature on any subject whatever well realizes the flagrant lack of adequate compedia. In sponsoring the sweet-potato- and cranberry-disease monographs, the United States Department of Agriculture has performed a gracious act for which pathologists, regardless of where they may be, should be duly thankful.

No less appreciative of Messrs. Shear, Stevens, and Bain should be their fellow workers, for they have issued a work that may well serve as a model for later efforts.—W. A. WHITNEY, Washington, D. C.

Acta Phaenologica, an international bimonthly journal of phenology sponsored by the *Nederlandsche Phaenologische Vereeniging,* and published by Martinus Nijhoff, The Hague. Beginning with the year 1932.

Adding another outlet of publication to the already oversupplied field of natural science is a task that may be approached with fear and trembling. However, there are branches of research that up to the present time are without an adequate organ for the dissemination of their results: a journal that will be particularly adapted for and devoted primarily to a restricted field. Accordingly, *Acta Phaenologica* is welcomed into the family of scientific publications.

Sponsored by the *Nederlandsche Phaenologische Vereeniging* under the secretarial supervision of Dr. H. Bos of that institution, the *Acta* appears as a bimonthly record of phenological research, open to any worker whether or not affiliated with the Dutch society.

In offering the initial number, the editor outlines his policy:

The periodical will welcome articles of different character and substance. Several will be original works . . . others less detailed. . . . We will insert also summaries of articles which have appeared elsewhere, either in other periodicals or in books. . . . We shall attempt to give also bibliographical bulletins, including the appearing of annual phenological reports. . . . Moreover, we intend to announce problems which call for general collaboration, observation, methods of operating, and notation. We shall welcome questions and answers, "personalia," desiderata, offers regarding the borrowing and exchanging of objects, manuscripts, books, statistics, etc., and finally suggestions, concerning the form, editorship, and organization proper, on condition that discussion of these last points be deferred until after completion of the first volume.

Naturally with so wide a scope and purpose, the happy culmination of the sponsor's dream is more than ordinarily dependent upon the wholehearted cooperation of all interested parties. So worthy a publication should surely receive support, and I, in the editor's language, heartily "urge everyone who is convinced of the necessity of a consolidation of phenological material to subscribe immediately, so that the publisher, at the end of the trial-period of three years, need not be obliged to discontinue the publication, and we express the hope that at the end of this period the new journal may be firmly established."

The first number of the *Acta Phaenologica* is one of 40 pages, of which 9 and a fraction are taken by the editor's introduction, with French and German renditions of the English. Dr. H. Bos outlines the *"Begriff und Zukunft der Phaenologie"* (pp. 11–22). "The Cold Spring of 1929 in the British Isles. I. Reaction by Insects and Birds," by J. Edmund Clarke, comprises pages 23 to 28. S. Illichevsky of the Union of Soviet Socialist Republics describes "The Results of the Phenological Observations at

Poltava'' (p. 29–37). In a short note J. C. Pfeiffer reports on *''Phaenologie et Mauvaises Herbes''* (p. 38). The last 2 pages (39 and 40) are taken up with an author abstract of E. Hiltner's *''Die Phänologie und ihre Bedeutung für die Klimatische Behandlung der Pollenallergie''* in Gutman's *''Die Pollenallergie; Heuschnupfen, Heufieber, Heuasthma.''*

In format the *Acta Phaenologica* presents a creditable appearance, its type-page, $6\frac{3}{4}$ x $4\frac{1}{8}$ inches, a clear type face, and a not-too-glossy paper combining to make a page very easily read. Though without half-tones or cuts of any kind, there seems to be no reason why the illustrations of later numbers should not be well reproduced, of course, with the natural proviso that the originals are worthy.

Of particular interest and importance are the language requirements—or lack of them—in the journal. Articles are accepted when written in English, French, or German, provided summaries in the other two languages also are submitted. This requirement might well be copied in other journals of ''international'' character. Such summaries will add immensely to the use of the journal in every land, offering to the unilingual scientist (and, in spite of language requirements in advanced university training, there are many of them!) a wider acquaintance with the literature.

Whether the *Acta Phaenologica* fills an essential gap in scientific periodicals or whether the papers within the scope of the journal might as fittingly—if not actually more so—be printed in a publication more closely allied to the subject whose climatic relations are discussed is a moot question, the definite answer to which must of necessity be determined by the individual author as each case arises. For example, a paper treating of the epidemiology of *Colletotrichum lindemuthianum* might better be issued in PHYTOPATHOLOGY or some other phytopathological journal, thus reaching an audience having somewhat more than an academic interest in the phenological phenomena presented.

On the other hand, however, there are papers whose theme and treatment are phenological first, with other considerations coming later. For such papers there has been no logical place in which to publish. Because of this, it is not to be inferred by the previous statement that the *Acta Phaenologica* is superfluous: it most decidedly is not, and, complementing other journals, it closes the hiatus in the literature sources between natural sciences *per se,* on the one hand, and pure meteorology, on the other.

In recent years the consideration of the relation of weather to the occurrence of plant phenomena in general and diseases in particular has come increasingly to the fore, yet, despite these advances, especially in the epidemiology of cereal rusts that have been made, the potentialities of the field are as yet unrealized. The appearance of the *Acta Phaenologica* can not fail to further the advances in its line by furnishing not only a logical but a highly commendable place for publication.—W. A. WHITNEY, Washington, D. C.

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THE NATURE OF POTATO RUGOSE MOSAIC

KARL LEE KOCH[1]

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INTRODUCTION

Within the past 15 years the potato has been shown to be subject to many different virus diseases. That some of these, however, might be due to a complex of 2 or more distinct viruses has been recognized for some time. Recent investigations showing that some potato viruses are transmissible to related host plants have stimulated closer attention to the viruses themselves as distinct entities. Additional methods for separating plant viruses have enabled a more careful scrutiny of the potato-virus diseases for possible virus combinations, which may occur in nature as typical diseases. Potato rugose mosaic, especially, was a source of considerable speculation relative to its nature and behavior. This disease is so constituted that it is almost as obscure as any infectious disease with which plants are affected. The nature of the disease has, therefore, come to light only very gradually through the contributions of several different workers. The present investigation of rugose mosaic was started, and its dual nature established, before it was known that other workers had arrived at and published similar conclusions. Several phases were in need of clarification, however, and with this object in view the problem was expanded and continued, the results of which are here presented.

REVIEW OF LITERATURE

Atanasoff's (3) review of early descriptions of potato diseases of the type that are now believed to have been of virus origin indicates that this group of maladies has been recognized for some time. As far as can be clearly established from the literature, however, the conception of the virus nature of these potato diseases was first developed in 1914 by Orton (13) and Appel (2). It remained, however, for Schultz, Folsom, Hildebrandt,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

The writer wishes to acknowledge his indebtedness to Dr. James Johnson for the opportunity to conduct these investigations under his direction and in his laboratory. He wishes to express his appreciation to Prof. H. F. Wilson, who has made possible the carrying on of this work.

and Hawkins (20) in 1919 to prove conclusively the relation of viruses to potato degeneration diseases. It soon became evident to Schultz and his coworkers (19) and almost simultaneously to Murphy (12) in Ireland and Quanjer (15) in Holland that several different viruses were concerned in these obscure potato maladies.

What may be termed the second period in the potato-virus problem became a study of the symptomatology of the diseases themselves, to which many investigators contributed. The particular disease with which we are concerned in this paper was named rugose mosaic by Schultz and Folsom (19) and was described as follows: "More infectious than 'mild mosaic' and differentiated by distinct dwarfing, more chlorosis and diffuse mottling, a more rugose type of wrinkling, and a tendency to show brittleness, spotting, streaking, leaf-dropping and premature death. Tuber symptoms are a marked reduction in size." What is probably the same disease was called crinkle by Murphy (11) and Quanjer (14).

The third period of development, regarding rugose mosaic, may be said to have started in 1925 when Johnson (7) demonstrated the presence of a virus in all apparently healthy potatoes of most standard American varieties. This virus known as the mottle virus was, however, sometimes associated in apparently healthy potatoes with what appeared on the basis of symptoms to be another virus, named by Johnson ring-spot (7). Johnson's results indicated that the mottle virus could be increased in virulence by passage through tobacco, producing a "spot-necrosis" disease of tobacco and a severe disease of potatoes. This spot-necrosis of tobacco was later recognized by him (9) to be identical with potato rugose mosaic.

It was at this point that the writer (10) took up the studies in Johnson's laboratory and showed definitely that there were actually 2 distinct viruses concerned in rugose mosaic of potato or spot-necrosis of tobacco, the one being the mottle virus present in apparently healthy potatoes and the other an insect-transmitted virus.

However, it soon became evident that, prior to the writer's studies, K. M. Smith (21), in England, had worked along the same lines with a disease referred to as potato mosaic, although he did not recognize the dual nature of this disease until 1931 (22).

Valleau and Johnson (23), of Kentucky, working at about the same time as Smith, claimed to have separated the 2 viruses producing rugose mosaic through the medium of nitrogen-starved tobacco plants and that the 2 viruses were found independently in nature. They recognized one of these to be the same as Smith's insect-transmitted virus, although they apparently did not carry on experiments in aphid transmission. Smith did not apply a satisfactory name to his insect-transmitted virus, and, as Valleau and Johnson applied the name veinbanding virus to this entity,

the use of this name may serve as well as any other, unless questions of priority in nomenclature arise.

The recent related work of others, such as that of Salaman (17, 18) and Quanjer (16), should also be taken into consideration. The puzzling potato-virus combinations and their differentiation are given extensive consideration, but, until more is known about the identity of the particular viruses used by different workers, it is not easy to correlate their work with that of others on the basis of published results only.

EXPERIMENTAL METHODS

The experiments on potato rugose mosaic were conducted under greenhouse conditions during the fall, winter, and spring months, as the environment and other conditions of the experiments can be more carefully controlled than in the field. Two greenhouses, one running at a lower temperature (17–22° C.) and one at a higher (27–32° C.), were available for the work.

The Bliss Triumph variety of potato was used in almost all experiments as the standard stock. The tubers were from indexed stocks, essentially free from the common virus diseases, though they all carried the mottle virus, normally present in apparently healthy American potatoes. The potatoes were grown in the cool house in rich compost soil and produced short, stocky, vigorous plants used for inoculation purposes.

The Connecticut Havana No. 38 strain of tobacco was used as the standard variety in all experiments. The plants were grown in the warm greenhouse, where the symptoms were produced more readily than in the cool house. Other solanaceous hosts were also grown in the high-temperature house. The tobacco was grown in new compost soil in 4-in. pots. To insure against any virus contamination from the pots themselves, they were previously steam-sterilized. The greenhouses were fumigated once a week to guard against aphid infestation.

The inoculum was obtained by crushing foliage of infected plants in a small sterile mortar, then straining the juice through cheesecloth. The potato plants were inoculated, when 2 to 5 in. high, by rubbing the upper surfaces of 3 young leaflets with a small piece of cheesecloth soaked in the prepared inoculum. A combination of the rubbing and pricking methods was used for inoculating the tobacco plants. This was done with a sterile needle, wrapped at the end with a wad of absorbent cotton to aid in carrying the inoculum, by pricking the 2 oldest leaves of the plant several times along the midrib and then rubbing or wiping the entire upper surface of the 2 leaves with the same needle. Other solanaceous hosts, excluding the potato, were inoculated and treated in the same manner as were the tobacco plants.

For insect-transmission experiments, the peach aphid, *Myzus persicae* Sulz., and the green potato aphid, *Macrosiphum solanifolii* Ashmead, were used as vectors. The green potato aphids were colonized upon young potato plants, while the peach aphids were colonized upon cabbage plants, suitable hosts for the aphids. As has been shown by Smith (22), as well as by our own trials, the aphids do not transmit the mottle or ring-spot viruses from potato, and there is no reason to believe that cabbage is susceptible to any of the viruses used. The method followed in these experiments was similar to that used by Hoggan (6).

The properties of the viruses were determined by using the methods employed by Allard (1) and Johnson (8). The inoculum used in these studies was obtained from infected tobacco plants.

EXPERIMENTAL RESULTS

Separation of the Viruses Causing Rugose Mosaic. The transmission of rugose mosaic (Fig. 1, B) by aphids was first reported by Schultz and

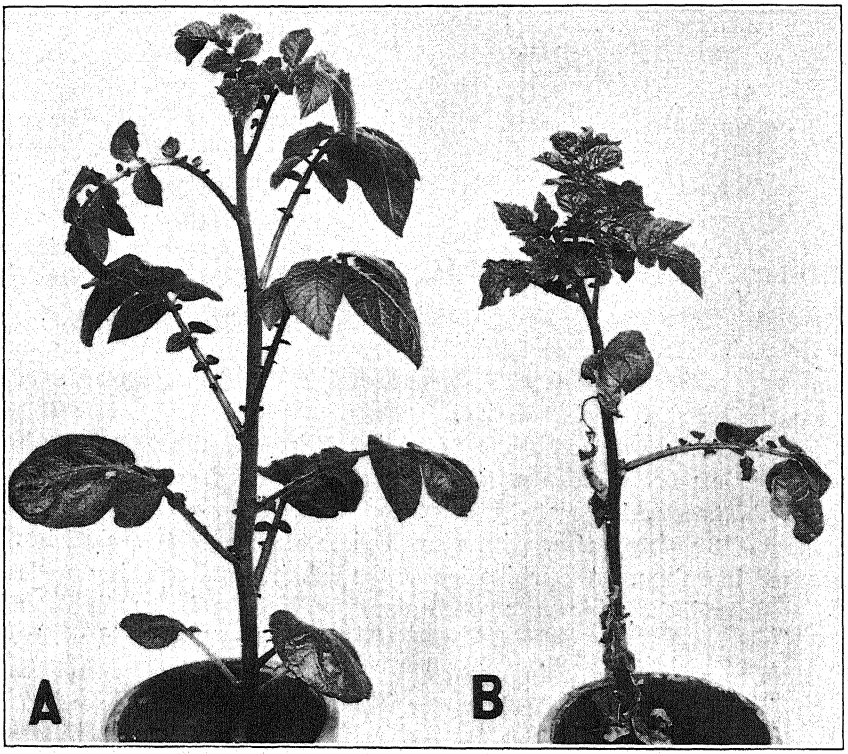


FIG. 1. Bliss Triumph variety of potato. A. Noninoculated control. B. Affected by rugose mosaic.

Folsom (19), who obtained apparent transmission of this disease by aphids from potato to potato. In the present investigation the writer also has readily obtained apparent transmission of this disease from potato to potato by means of the aphids *Myzus persicae* and *Macrosiphum solanifolii* (Table 1). Apparent transmission of the disease to potato was obtained

TABLE 1.—*Summarized results comparing aphid and artificial transmission of potato rugose mosaic to potato and tobacco*

Host inoculated	Source of inoculum	Inoculation by	No. of plants inoculated	No. developing symptoms of		Uninoculated controls	
				Rugose mosaic or spot-necrosis	Vein-banding only	No. of plants	No. devel. symptoms
Potato	Rugose mosaic on potato	Plant extract	15	13	0	5	0
		Myzus persicae	93	57	0	90	0
		Macrosiphum solanifolii	95	25	0	90	0
	Spot-necrosis on tobacco	Plant extract	40	33	0	25	0
		Myzus persicae	26	19	0	20	0
Tobacco	Rugose mosaic on potato	Plant extract	60	45	0	10	0
		Myzus persicae	29	0	27	25	0
		Macrosiphum solanifolii	38	0	23	35	0
	Spot-necrosis on tobacco	Plant extract	60	59	0	25	0
		Myzus persicae	35	0	35	30	0

also from tobacco by means of *Myzus persicae.* However, when an attempt was made to transmit rugose mosaic by means of aphids to tobacco, from either potato or tobacco, none of the aphid-infested tobacco plants developed symptoms of spot-necrosis (Table 1). These plants, however, were abnormal. They developed faint symptoms of another type, which appeared mainly as a clearing of the veins and a general flattening of the plants. Such symptoms were characteristic of all tobacco plants similarly infested

with aphids from rugose mosaic hosts. As these symptoms are later shown to be caused by a virus, similar to the veinbanding virus described by Valleau and Johnson (23), this disease will be referred to in this paper as veinbanding (Fig. 2, D).

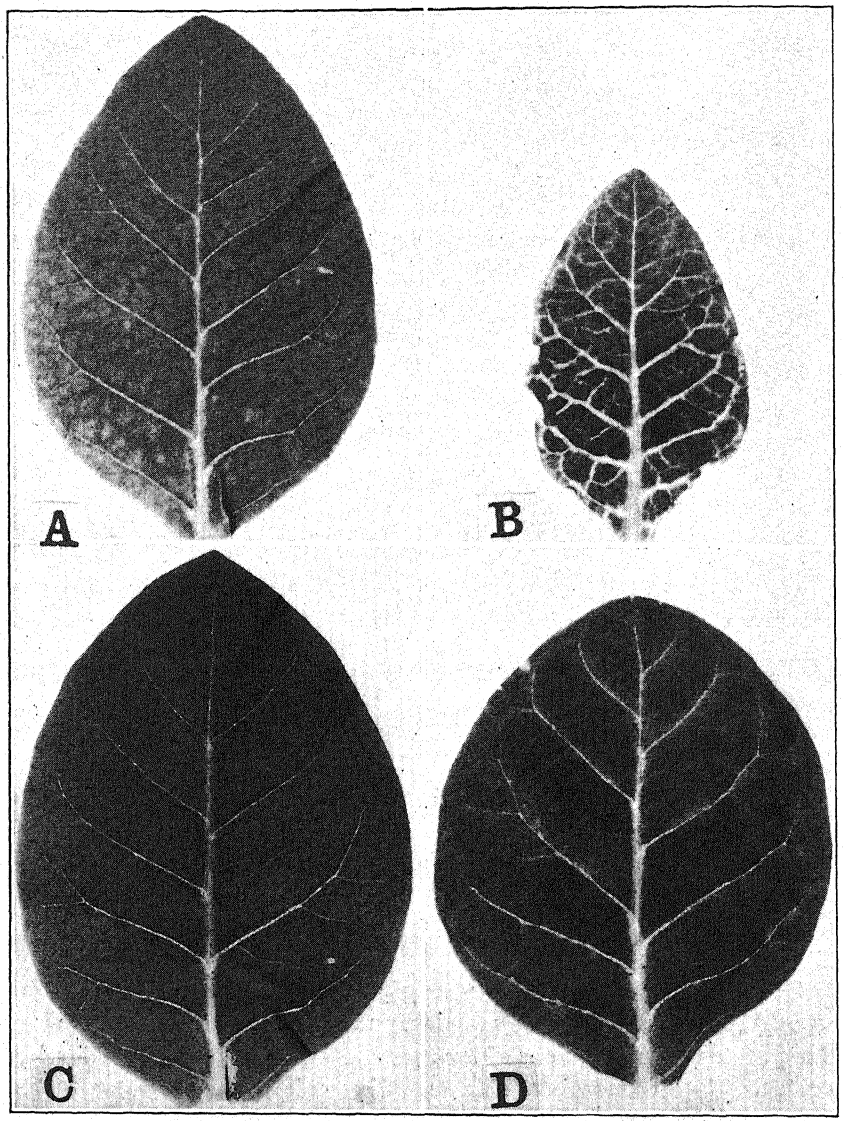


FIG. 2. Comparison of symptoms produced on tobacco by mottle and veinbanding viruses. A. Mottle. B. Spot-necrosis produced by a mixture of the two viruses. C. Control. D. Veinbanding.

These experiments suggested that 2 distinct viruses might be associated with the disease, both of which are readily transmissible by artificial methods, while only one of them is transmitted by aphids, *i.e.*, the veinbanding virus. That the other is the mottle virus (Fig. 2, A), normally present in apparently healthy potatoes, was suggested by Johnson's earlier work on this virus (7, 9).

The veinbanding virus proved readily transmissible from tobacco to tobacco both by aphids and by plant extract (Table 2). That this virus

TABLE 2.—*Summary of results on transmission of the veinbanding virus to tobacco*

Inoculation by	Source of inoculum	No. of plants inoculated	No. of plants showing veinbanding symptoms
Plant extract	Apparently healthy potatoes	80	0
	Veinbanding on tobacco	100	100
Aphids (*Myzus persicae*)	Apparently healthy potatoes	20	0
	Veinbanding on tobacco	17	14

was not present in the apparently healthy potatoes used in the experiments was shown by the fact that it was not recovered from such potatoes by either method of transfer (Table 2). Failure of the aphids to transmit the mottle virus, either from potato or tobacco, is shown in table 3. As shown

TABLE 3.—*Summary of results on transmission of the mottle virus to tobacco*

Inoculation by	Source of inoculum	No. of plants inoculated	No. of plants showing mottle symptoms
Plant extract	Apparently healthy potatoes	80	75
	Mottle on tobacco	75	74
Aphids (*Myzus persicae*)	Apparently healthy potatoes	20	0
	Mottle on tobacco	35	0

in the table, however, this virus may readily be obtained on tobacco free from the veinbanding virus by artificial inoculation of extract from apparently healthy potatoes.

Recombination of the Viruses Causing Rugose Mosaic. Since 2 distinct viruses, which can be maintained separately in tobacco, were obtained from rugose mosaic plants, the next step was to recombine them in tobacco to determine whether spot-necrosis (rugose mosaic) would then develop. Three series of tobacco plants were, therefore, inoculated with different methods of combination of the veinbanding and mottle viruses. As shown in table 4, all tobacco plants inoculated with these mixtures developed spot-

TABLE 4.—*Symptoms developing on tobacco as a result of artificial inoculation with the mottle virus and the veinbanding virus, separately and in combination*

Viruses	No. of plants inoculated	Symptoms		
		Mottle	Veinbanding	Spot-necrosis
Mottle	15	15	0	0
Veinbanding	15	0	15	0
Veinbanding and mottle viruses inoculated separately to each plant	25	0	0	25
Veinbanding and mottle viruses combined before inoculation	20	0	0	20
Veinbanding virus inoculated to plants previously infected with the mottle virus	20	0	0	20
Controls	15	0	0	0

necrosis (Fig. 2, B), while those inoculated with either virus alone did not, thus showing that symptoms of spot-necrosis may be produced by a combination of veinbanding and mottle.

Further evidence that the veinbanding virus is one factor in the production of rugose mosaic of potato is given in table 5. Here it is shown that 25 out of 26 apparently healthy potatoes, which normally contain the mottle virus, developed symptoms of rugose mosaic when inoculated with the veinbanding virus alone. To demonstrate that the mottle virus is the other factor essential for the production of this disease on potato, 25 potato seedlings, which, according to Johnson (7), are mottle-free, were inoculated with the veinbanding virus alone, 8 with a mixture of the mottle and veinbanding viruses, and 3 with plant extract obtained from a rugose-mosaic potato plant. As shown in table 5, the 3 seedings inoculated with rugose mosaic, as well as the 8 inoculated with the mixture of viruses, all developed

TABLE 5.—*Comparative inoculations on apparently healthy potatoes, and on seedling potatoes free from the mottle virus*

Host inoculated	Inoculum	No. of plants inoculated	No. of plants developing rugose mosaic
Apparently healthy potatoes	Veinbanding	26	25
Seedling potatoes	Veinbanding	25	0
	Veinbanding and mottle combined	8	8
	Rugose mosaic	3	3

symptoms of rugose mosaic. The 25 seedlings inoculated with the veinbanding virus alone, however, did not develop symptoms of rugose mosaic; for, although a slight rugosity and mottling of the upper leaves occurred, there was no basal leaf necrosis nor leaf drop. Since the veinbanding virus alone did not cause rugose mosaic in seedling potatoes, it is concluded that the mottle virus, as well as the veinbanding virus, must be present in order to produce typical symptoms of the disease.

The Separation of the Mottle-ring-spot Complex. With the mottle virus in apparently healthy potatoes, there is sometimes found a virus described by Johnson (7) as ring-spot (Fig. 3, C). The question arises as to what part ring-spot plays in the virus complex of spot-necrosis (rugose mosaic). To investigate this relationship it was necessary to obtain ring-spot free from mottle, a condition, it is believed, that has not previously been accomplished.

Following the discovery of 2 viruses in apparently healthy potatoes by Johnson (7) there have been many different names applied to these viruses. Johnson used the names mottle and ring-spot as being descriptive of the symptoms occurring on tobacco, it then not being known whether these viruses existed as such in the potato. It seems quite evident that Valleau and E. M. Johnson (23) included these 2 viruses in what they call "the healthy potato virus"; although, according to their descriptions and illustrations, they obtained symptoms identical with those produced by both mottle and ring-spot. While recognizing these 2 viruses as described by Johnson (7), Burnett and Jones (4) have given them different names, calling one the latent virus and the other the virulent latent virus. In England, Smith (22) describes the symptoms produced by a virus which he calls "ring-spot." This appears to be similar to the potato ring-spot described by Johnson (7). He does not describe a virus similar to mottle, however, and it is not possible from the published descriptions to determine if this

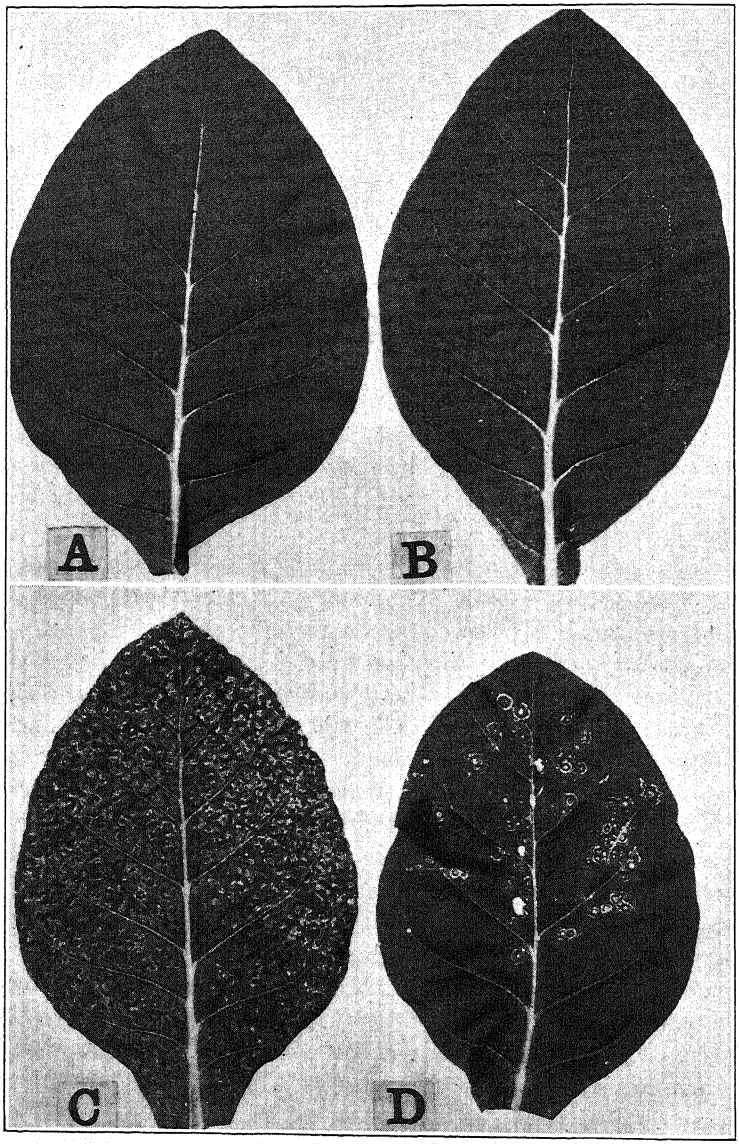


FIG. 3. Comparison of symptoms produced on tobacco by mottle and ring-spot viruses. A. Control. B. Mottle. C. Ring-spot. D. Local lesions produced by ring-spot.

virus was present in combination with his ring-spot. In Holland, Quanjer (16) has worked with a virus that he states is known to be latent in the American varieties Green Mountain, Irish Cobbler, and Rose. Quanjer is

evidently referring to one or both of the viruses found in the apparently healthy American potatoes.

Mention should be made that the ring-spot obtained from apparently healthy potatoes should not be confused with another ring-spot disease of tobacco, first shown by Fromme, Wingard, and Priode (5) to be due to a virus.

Since both the mottle and the ring-spot viruses are found in apparently healthy potatoes, especially in the variety Rural, there has been some question as to their identity as separate viruses. Following his first description of the symptoms produced on tobacco, Johnson (7) expressed the opinion that 2 distinct viruses were probably concerned, although he did not actually separate them on the basis of properties or in any other way. There is little doubt that the mottle virus has often been obtained upon tobacco from apparently healthy potato plants free from the ring-spot virus, since mottle is regularly present, while ring-spot appears to be much less common. So far as can be ascertained, however, there is no indication in the literature that the potato ring-spot virus has been separated from the mottle virus and propagated in a "pure" form.

During the course of the experiments, it was observed that the first symptoms of ring-spot appeared on tobacco about 3 days after inoculation, while the first symptoms of mottle did not appear until about 7 days after inoculation. This suggested a possible difference in the rate of progress of the 2 viruses through the tobacco plant, by means of which their separation might be accomplished. The lowest leaves of young tobacco plants were, therefore, inoculated with a mixture of mottle and ring-spot, and it was found possible by means of inoculations from the tips of these plants at intervals of 6 or 12 hours to obtain what appeared to be pure ring-spot upon tobacco (Table 6). In Series I inoculations were made every 12 hours from the tips of the original plants, the first inoculation being made 12 hours after the introduction of the virus mixture into the plants. Ring-spot was recovered in this series 96 hours after the original inoculation, while mottle was not recovered until after 108 hours. In Series II and III inoculations from the tips of the young leaves were made every 6 hours but were not begun until 66 hours after the introduction of the virus mixture. Ring-spot was first obtained in Series II 72 hours after the original inoculation, although mottle was not recovered until 108 hours had elapsed. Series III did not yield ring-spot until after 84 hours, while mottle was secured in 102 hours.

To determine if the mottle virus was still associated with the ring-spot virus obtained in this manner, property determinations were made. Since, as will be shown later, the ring-spot virus is slightly less resistant to heat, dilution, chemicals, and aging than is the mottle virus, mottle, if

TABLE 6.—*Results of experiments planned to separate the ring-spot and mottle viruses on the basis of their rates of progress through tobacco plants*

Number of hours allowed for viruses to pass through plant	No. of plants inoculated in each series	Series I		Series II		Series III	
		No. of plants showing ring spot	No. of plants showing mottle	No. of plants showing ring spot	No. of plants showing mottle	No. of plants showing ring spot	No. of plants showing mottle
66	5	0	0	0	0	0	0
72	5	0	0	1	0	0	0
78	5	–	–	0	0	0	0
84	5	0	0	3	0	4	0
90	5	–	–	0	0	3	0
96	5	1	0	4	0	5	0
102	5	–	–	1	0	4	2
108	5	4	1?	5	2	5	3
114	5	–	–	5	3	–	–
120	5	4	1	5	5	–	–

present, should appear in tests designed to eliminate ring-spot but not mottle. The ring-spot virus will tolerate dilution of 1 to 10,000 but not 1 to 100,000, and, although mottle will tolerate a dilution of 1 to 100,000, it did not appear at this dilution of the ring-spot virus in any of the trials. Also, in testing the effect of heat on the virus, it was found that after heating at 68° C., a temperature that inactivates ring-spot but not mottle, no mottle developed. In addition, it was found that, although ring-spot is not so resistant to alcohol as is the mottle virus, mottle did not appear where it might be expected to do so if present in the virus extract. From these tests it was concluded that the ring-spot virus obtained in the manner described above was free from the mottle virus and that the 2 viruses are actually distinct entities.

It is particularly worthy of note that a combination of the ring-spot and veinbanding viruses produced symptoms on tobacco very similar to the spot-necrosis produced by a combination of the mottle and veinbanding viruses (Fig. 4). Spot-necrosis symptoms of a severe type were produced in 5 separate trials on a total of 25 tobacco plants by inoculating them with a combination of the ring-spot and the veinbanding viruses. Furthermore, the type of spot-necrosis produced by the ring-spot-veinbanding combination was found to develop necrosis of the veins earlier and to produce a

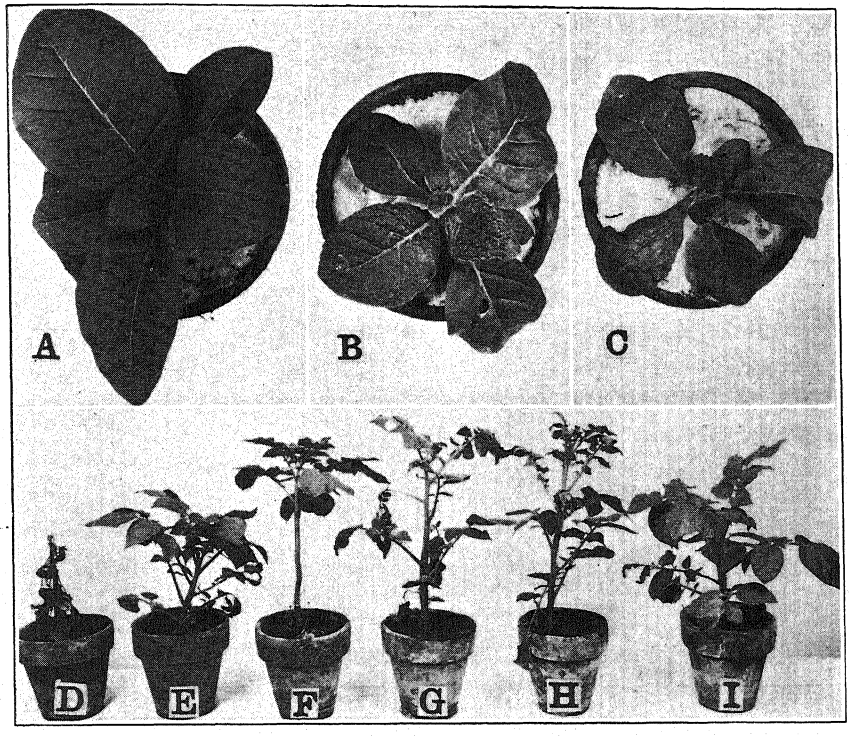


FIG. 4. A–C. Severity of spot-necrosis produced on tobacco by different combinations of viruses. A. Control. B. Combination of veinbanding and mottle viruses. C. Combination of veinbanding and ring-spot viruses. D–I. Symptoms of rugose mosaic produced on 6 common American varieties of potatoes by veinbanding virus. D. Bliss Triumph. E. Early Ohio. F. Green Mountain. G. Irish Cobbler. H. Rural New Yorker. I. Early Rose.

severer type of necrosis than did the mottle-veinbanding combination. Although the 2 viruses, when combined with veinbanding, differ in the severity of spot-necrosis symptoms produced, they are strikingly alike in general behavior. Since, however, the mottle virus is usually present in American varieties of potatoes, while ring-spot is not so common, it is evident that under field conditions the former virus is likely to be the most important in the production of rugose mosaic.

THE MOTTLE VIRUS

The mottle virus used in these experiments has been identified by James Johnson as identical with that described by him in 1925 (7). The presence of this virus in Havana tobacco is indicated by the darker green tissue along the veins, while that between these darkened lines becomes slightly chlor-

otic. Necrotic lines and rings are entirely absent. (Figs. 2, A and 3, B). Some variation in the degree of severity, however, may occur.

TABLE 7.—*A comparison of the thermal death points of the veinbanding, ring-spot, and mottle viruses*

Temperature	Number of plants infected out of 20 inoculated		
	Veinbanding	Ring spot	Mottle
°C.			
50	20		
54	19		
55	17		
56	4		
58	1		
60	0	20	20
65		19	20
68		0	7
70		0	0

TABLE 8.—*The effect of aging upon the infectivity of the veinbanding, ring-spot, and mottle viruses*

Aging of virus	Number of plants infected out of 20 inoculated		
	Veinbanding	Ring spot	Mottle
Days			
None	17	20	20
2	9	20	20
3	8	—	—
4	7	17	20
5	3	—	—
6	0	11	20
8	0	14	19
10		12	19
12		12	20
14		17	20
16		9	18
18		9	14
20		12	15
22		10	13
24		13	13
26		14	10
28		7	9

TABLE 9.—*The effect of dilution upon the infectivity of the veinbanding, ring-spot, and mottle viruses*

Dilution	Number of plants infected out of 20 inoculated		
	Veinbanding	Ring spot	Mottle
None	20	20	20
1–100	20	20	20
1–500	18	—	—
1–1,000	12	19	20
1–5,000	4	—	—
1–10,000	0	12	16
1–100,000	0	0	8
1–1,000,000	—	0	0

TABLE 10.—*The influence of various chemicals upon the infectivity of the veinbanding, ring-spot, and mottle viruses*

Chemical	Strength of chemical	Number of plants infected out of 20 inoculated					
		Veinbanding		Ring spot		Mottle	
		30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
Nitric acid c. p.	1–500	0	0	20	20	20	19
Formaldehyde (37%)	1–100	1	0	16	10	13	10
Alcohol (absolute)	25%	19	16	20	20	20	20
Alcohol (absolute)	50%	1	0	7	6	17	15

The virus is readily transmitted by artificial means, but not by aphids. Failure of aphids to transmit the mottle virus has already been demonstrated in this paper. The mottle virus may readily be obtained on tobacco by artificial inoculation from apparently healthy potatoes of most American varieties or by separation from veinbanding on the basis of its greater resistance to heat and other treatments.

Property studies on the mottle virus show that it will withstand heating, aging, dilution, and chemicals to a greater degree than will either the ring-spot or veinbanding virus (Tables 7–10). This virus is inactivated at a

temperature of 70° C. It will tolerate a dilution of 1 to 100,000, though it is not infectious at a dilution of 1 to 1,000,000. After being subjected to aging for 28 days at room temperature, mottle may be still highly infective. It is only slightly affected by 60-minute exposures to nitric acid (1 to 500), formaldehyde 37 per cent (1 to 100), or 50 per cent alcohol.

THE RING-SPOT VIRUS

The first symptoms of potato ring-spot on Havana tobacco appear 3 to 4 days after inoculation as small local lesions on the inoculated leaves. (Fig. 3, D). These lesions at first consist of a small central necrotic spot around which occur 1 or 2 necrotic rings. Later, the number of rings increases until there may be several in a single lesion. Symptoms on the upper leaves, which appear 6 to 7 days after inoculation, consist of irregular necrotic lines and concentric rings, which often coalesce into large necrotic areas. (Fig. 3, C).

Ring-spot is readily transmitted by artificial means. It does not appear, however, to be transmissible by the aphid *Myzus persicae,* since no infection was obtained in 8 separate trials involving a total of 40 tobacco plants infested with aphids that had previously fed upon ring-spot-infected tobacco plants.

Property studies upon the ring-spot virus show that it is not quite so resistant as the mottle virus, though more so than the veinbanding virus (Tables 7–10). Ring-spot is inactivated at a temperature of 68° C. It is still infectious after aging for 28 days, though its infectivity has apparently been reduced. This virus will tolerate a dilution of 1 to 10,000 but is not infective when diluted 1 to 100,000. Ring-spot is only slightly affected by a 60-minute exposure to nitric acid (1–500) and formaldehyde 37 per cent (1 to 100). It is, however, rendered less infectious by a 60-minute exposure to 50 per cent alcohol.

THE VEINBANDING VIRUS

As the experiments in the University of Wisconsin have been almost entirely confined to the Havana-Seed type of tobacco, a comparison was made of the symptoms produced by veinbanding on this and other distinct types of *Nicotiana tabacum.* The Burley, Orinoco, Turkish, and Pryor types were chosen in addition to Havana Seed for this comparison. The Havana-Seed, Orinoco, Burley, and Pryor types produced symptoms of veinbanding, consisting of a general flattening of the entire plant, a slight clearing of the veins, and a faint chlorosis of the leaves (Fig. 2, D). Although the Turkish variety at first developed symptoms identical with those produced on the other types, a mottling later developed that was very similar to the symptoms caused by the mottle virus obtained from apparently healthy potatoes.

The veinbanding virus is readily transmitted by the aphids *Myzus persicae* and *Macrosiphum solanifolii,* as well as by plant extract. It may be obtained from the rugose mosaic or spot-necrosis complex by means of these insects.

Property studies upon the veinbanding virus show that it is less resistant than either the mottle or the ring-spot virus (Tables 7–10). Veinbanding is inactivated at a temperature of 60° C. It will withstand aging for only 4 or 5 days. This virus will tolerate dilution to 1 to 5,000 but not 1 to 10,000. Veinbanding will not withstand exposure to nitric acid (1 to 500), formaldehyde 37 per cent (1 to 100), or 50 per cent alcohol for 30-minute periods, although it is still infectious after being exposed to 25 per cent alcohol for 60 minutes.

Host Range. To determine a partial host range for the veinbanding virus, several species of solanaceous plants were selected and infested with aphids that had previously fed on rugose-mosaic potato plants. The infestations were made in series of 5 plants each, the juice from all 5 being subsequently combined and inoculated back to tobacco as a test for susceptibility of the host in question. If symptoms of veinbanding appeared upon the latter plants, it was evident that at least 1 plant in the series had been infected.

TABLE 11.—*A test of certain solanaceous plants as possible hosts of the veinbanding virus*

Plants inoculated	No. of series of 5 plants each infested with viruliferous *Myzus persicae*	Symptoms produced	No. of series producing veinbanding upon reinoculation to tobacco
Datura stramonium	4	None	0
Lycopersicon esculentum	6	Vein-clearing	6
Nicandra physaloides	6	None	6
Nicotiana glutinosa	4	Vein-clearing	4
" *rustica*	6	"	6
Physalis pubescens	6	None	6
" *longifolium*	4	"	0
Solanum minuatum	6	"	5
" *melongena*	6	"	0
" *nigrum*	6	"	5
" *carolinense*	4	"	0

It is shown in table 11 that *Lycopersicon esculentum, Nicandra physaloides, Solanum minuatum, S. nigrum, Nicotiana glutinosa, N. rustica,* and *Physalis pubescens* are susceptible to veinbanding since the virus was readily recoverable from infested plants by artificial inoculation to tobacco. On the other hand, no virus was recovered from *Datura stramonium, Physalis longifolium, Solanum melongena,* and *S. carolinense.* Similar results were obtained with *D. stramonium* by Valleau and Johnson (23) and possibly also by K. M. Smith (22), who failed to infect this species with his aphid-transmitted virus. The fact that certain solanaceous plants will act as hosts for veinbanding may be of some practical importance. It seems, as suggested by Valleau and Johnson (23), that certain solanaceous weeds growing near a potato field may possibly become infected with this virus and act as a source of infection for neighboring potato plants that already carry the mottle virus, resulting in the development of rugose mosaic.

Resistance in Potatoes. Johnson (9) has found that a marked difference in susceptibility exists among the common American potato varieties to several potato-virus diseases, including rugose mosaic. As rugose mosaic is now shown to be due to a combination of the veinbanding virus with one or both of the viruses found in the apparently healthy potato, an attempt has been made to determine the degree of resistance exhibited by the common American potato varieties to the veinbanding virus.

The Green Mountain, Bliss Triumph, Early Rose, Early Ohio, Irish Cobbler, and Rural New Yorker varieties were used, 50 plants of each variety being artificially inoculated with the veinbanding virus (Table 12). The results indicate that these varieties may be classed as either susceptible or resistant. The Green Mountain and Bliss Triumph varieties belong in

TABLE 12.—*Relative resistance of certain potato varieties to rugose mosaic when inoculated with the veinbanding virus alone*

Variety tested	No. of plants inoculated	No. of plants infected	Average incubation period in days	Symptoms		
				Necrosis	Mottling	Rugosity
Green Mountain.....	50	50	17.6	Severe	Good	Severe
Bliss Triumph	50	46	19.4	Very severe	"	Very severe
Early Rose	50	40	30.4	Slight	Slight	Slight
Early Ohio	50	38	29.7	Good	None	None
Irish Cobbler	50	29	28.4	"	"	"
Rural New Yorker	50	21	28.8	Slight	Good	Good

the susceptible class, since, in both, the disease has a relatively short incubation period, high percentages of infection are secured, and severe symptoms of rugose mosaic develop (Fig. 4, D–I). Green Mountain is probably slightly more susceptible than Bliss Triumph, since a higher percentage of infection was obtained on the former and the incubation period was somewhat shorter, although the symptoms are more severe in Bliss Triumph. None of the Green Mountain potatoes were entirely destroyed by this disease, while about 25 per cent of the Bliss Triumph potatoes were killed.

The Early Rose, Early Ohio, Irish Cobbler, and Rural New Yorker varieties evidently belong in the resistant group (Fig. 4, D–I), with Early Rose the least resistant and Rural New Yorker the most resistant of the 4. While the incubation periods of the disease in these 4 varieties are almost the same, the varieties vary considerably in the percentage of infection obtained, the Rural variety yielding about half as many infected plants as does Early Rose, and Irish Cobbler about 75 per cent as many.

From the experiments carried on by Johnson (9) and by the writer it appears there is a decided variation in the resistance exhibited by the common American potato varieties to the veinbanding virus, suggesting possibilities for future control measures.

COMPARATIVE STUDIES WITH VIRUSES FROM FOREIGN SOURCES

One of the most important problems in connection with potato-virus diseases is the correlation of research in various countries with reference to the specific identity of the viruses studied. In most cases it has remained almost impossible on the basis of symptomatology definitely to identify a virus disease, much less a virus, and until this is done much uncertainty will exist in the literature. For the present an exchange of materials for study appears to offer the most promising line of attack. Through the courtesy of R. N. Salaman, H. M. Quanjer, E. S. Schultz, D. Folsom, and others, the Wisconsin laboratories have been able to secure various viruses for comparative study, in a preliminary way, with the rugose-mosaic viruses. It may be said that our rugose mosaic agrees with Schultz and Folsom's rugose mosaic, which, in turn, is presumed to be identical with potato crinkle in Europe, as described by Murphy (11) and by Quanjer (14).

Salaman (17), in England, states that a disease named by him "Crinkle 'A'" is due to a combination of viruses, one element of which is responsible for the crinkle-mosaic type of symptom and another for the streak type. Since he also considers potato streak to be the same as rugose mosaic, the two component viruses of rugose mosaic might be expected to be comparable with those concerned in the production of streak.

A study has been made upon Salaman's Crinkle A as occurring in the potato variety President. Tobacco plants, artificially inoculated with extract from President potatoes affected with Crinkle A developed a mottling that appeared similar to that produced by the mottle virus. When the veinbanding virus was introduced into tobacco plants containing this virus spot-necrosis resulted, indicating further that Crinkle A includes the mottle virus. That Crinkle A did not include the veinbanding virus was inferred from the fact that artificial inoculation to tobacco yielded symptoms of mottle only and not of spot-necrosis.

These results suggested the possibility that those potato varieties in which Crinkle A caused streak might contain the veinbanding virus, particularly if, according to Salaman, streak and rugose mosaic are identical. Three varieties of potato in which Crinkle A was said to produce streak and 3 in which it was said to produce crinkle mosaic were tested for the presence of both the veinbanding and the mottle viruses. This was accomplished by means of direct inoculation from potato to tobacco. Extract from these potatoes was combined also with the veinbanding virus in a further test of the presence or absence of mottle with the expectation that, if this virus were present, spot-necrosis would be produced. Similarly, another portion of extract was combined with the mottle virus and inoculated to tobacco to determine whether veinbanding were present.

The varieties Up-to-Date, Duke of York, and King Edward, which, according to Salaman (17), belong to the streak-producing group, showed no evidence of containing the veinbanding virus, while the presence of mottle virus was demonstrated only in the Duke of York variety. The varieties Arran Victory, President, and Abundance, said to develop crinkle mosaic following inoculation with Crinkle A, were shown to contain neither veinbanding nor mottle. From these results it, therefore, seems unlikely that Salaman's streak is identical with our rugose mosaic.

Preliminary studies made upon certain potato-virus diseases obtained from Holland from H. M. Quanjer indicate that some of these diseases may be caused, in part at least, by viruses similar to those associated with our rugose mosaic. The presence of the veinbanding virus in potato plants that, according to Quanjer, were infected with "acropetal necrosis" (16) was demonstrated by artificial inoculation of extract to tobacco, symptoms of veinbanding developing upon the inoculated tobacco plants. Additional evidence of the presence of the veinbanding but not the mottle virus in these potatoes was obtained by combining part of the extracted juice with the veinbanding virus and part with the mottle virus and subsequently inoculating each combination to a series of tobacco plants. Tobacco plants inoculated with the veinbanding combination developed symptoms of veinbanding only, indicating the absence of mottle from the original potato

plants, while plants inoculated with the mottle combination developed symptoms of spot-necrosis, demonstrating the presence of the veinbanding virus in the potatoes. This fact was confirmed by inoculation of Bliss Triumph potatoes, carrying mottle, with the extract from the original potato plants, which resulted in symptoms of severe rugose mosaic.

A series of potatoes that, according to Quanjer, were infected with "acronecrosis" were tested in a similar manner and were also found to contain the veinbanding but not the mottle virus. The mottle virus, on the other hand, was recovered from potatoes said by Quanjer to be infected with leaf roll. This virus, strangely enough, was not recovered from potatoes said to be carriers of the apparently healthy potato virus.

From these preliminary studies it may be concluded that the veinbanding and mottle viruses are both present in England and Holland, though the question of their relative importance in the field remains obscure.

DISCUSSION

The potato-virus-disease problem, as a whole, is greatly complicated by the presence of one or more viruses in apparently healthy American potatoes. The problem may be said to be complicated still further by the irregular association of these viruses with apparently healthy European stocks of potatoes. Nevertheless, as far as rugose mosaic is concerned, it is evident that the information that has been accumulating from several sources in recent years is serving to clarify a situation that is much more complex than had been realized. The results, as a whole, go to show that the symptom complex, known as rugose mosaic in America, must generally be due to the mottle and the veinbanding viruses acting in combination. It is quite as evident, however, that an almost identical symptom expression may result from the ring-spot and veinbanding complex either alone or in combination with the mottle virus. This evidence would suggest rather than preclude the possibility of still other viruses being concerned in similar expressions of disease. It is believed, however, that when potato diseases of the rugose-mosaic, crinkle, and streak types have been adequately studied in various parts of the world the causal agencies will be found to have much in common. Such studies must evidently be based particularly upon the isolation of the viruses concerned and upon determinations of their comparative properties, modes of transmission, host range, and other distinguishing characteristics rather than solely upon extensive descriptions of symptoms.

The increased knowledge concerning the nature of the rugose-mosaic disease suggests several possible lines of endeavor in the direction of control. Fortunately, when the viruses concerned are combined, the result is a disease that tends to be self-exterminating, even though the viruses by them-

selves are admirably adapted to self-perpetuation. The prevention of insect dissemination of a virus disease through insect control is not yet a promising line of control in the field. The elimination of the insect-transmitted virus through tuber indexing of the potato stock itself, seems most likely to succeed. Should it develop that other host plants actually harbor the veinbanding virus to any extent, this would no doubt offer added but perhaps not insurmountable difficulties to the propagation of virus-free stocks. Still another line of control is well worthy of careful consideration in districts where rugose mosaic is a serious disease. Manifestly, the elimination of the mottle virus, normally present in apparently healthy potatoes, would prevent the development of the more serious rugose-mosaic disease, even though veinbanding were prevalent. Since potatoes free from the mottle virus can apparently be found (4) and since this virus is not aphid-transmitted, it would seem to be only a routine matter to build up large stocks of seed potatoes of different varieties free from this constituent of the disease.

Lastly, the probability of utilizing existing varieties of resistant potatoes or of developing new strains resistant to one or more of the viruses concerned in rugose mosaic should not be overlooked.

SUMMARY

Further evidence is presented to show that the rugose-mosaic disease of potato is due to the combined effect of the mottle virus, normally present in apparently healthy potatoes, with the veinbanding virus. The potato ring-spot virus may, however, replace, with almost identical results, the mottle virus in the combination.

Both the mottle virus and the ring-spot virus are easily transmitted by plant extract, but all attempts to transmit these by means of aphids have failed. The veinbanding virus, which is also transmissible by plant extract, is readily transmitted as well by the aphids *Myzus persicae* and *Macrosiphum solanifolii*.

The differential behavior of these viruses with respect to insect transmission serves as a most simple means of isolating the veinbanding virus from the rugose-mosaic complex. The mottle virus, on the other hand, may be readily separated from the complex on the basis of differential properties.

The mottle virus, as secured either from the rugose-mosaic complex or from apparently healthy potatoes, may sometimes be combined with the potato ring-spot virus, while the latter has not yet been found except in association with mottle.

Mottle and ring-spot apparently can not be separated upon the basis of transmission or differential properties. It was found, however, that the

ring-spot virus could be isolated from mottle by utilizing the differential rate of progress of the 2 viruses through the tobacco plant.

The thermal death point of the veinbanding virus was found to be 60° C., that of ring-spot 68° C., and that of mottle 70° C. The veinbanding virus resists aging *in vitro* only about 5 days, whereas ring-spot and mottle may resist aging as long as 28 days. Veinbanding will withstand a dilution of only about 1 to 5,000, and ring-spot 1 to 10,000, whereas mottle will withstand a dilution as high as 1 to 100,000. Veinbanding is also more sensitive to chemical treatments than is ring-spot or mottle.

Tests on resistance to rugose mosaic in 6 common American varieties of potato indicate that this varies with the variety and, in the Rural New Yorker, at least, it is perhaps great enough to warrant use of this variety as a resistant potato.

Certain solanaceous plants are shown to be susceptible to the veinbanding virus. This suggests the possibility of aphids transmitting veinbanding from weed hosts to neighboring potato plants, which, since they already harbor the mottle virus, would then develop rugose mosaic.

Comparative studies with virus diseases from foreign sources indicate that viruses similar to or identical with mottle and veinbanding may be found in foreign potatoes. The existence of certain varieties or strains free from the mottle and ring-spot viruses suggests the use of such potato stocks as a possible method of control of rugose mosaic.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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PHYSIOLOGIC FORMS OF WHEAT STEM RUST IN KANSAS AND NEBRASKA[1]

GEORGE L. PELTIER[2]

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INTRODUCTION

The writer here presents a summary of an intensive study of the primary sources of wheat stem rust in Kansas and Nebraska, based on observations of the spread of rust and on the annual occurrence of the physiologic forms of *Puccinia graminis tritici* (Pers.) Eriks. and Henn. during a 3-year period (1928–30). The methods employed in making the surveys and collecting the material for the identification of the forms were essentially the same as those already described in detail by Peltier and Thiel (3).

Since the publication of this paper Stakman, Levine, and Wallace (5) and Wallace (6) have reported the physiologic forms identified by them in the United States and Mexico, but particularly those occurring in the Mississippi Valley over a 3-year period (1926–28). Recently, Newton, Johnson, and Brown (1) have summarized the physiologic forms of wheat rust found in Canada over an 11-year period (1919–29). Peltier (2), on the basis of 6 years' observations, has discussed some of the aspects of the spread of stem rust in Nebraska.

PHYSIOLOGIC FORMS FOUND IN 1928

Near Barberries. In Kansas, aeciospores were first noted the last week in May in Republic, Saline, and Riley counties. Uredinia were first observed on grasses near infected bushes on June 6, 13, and 14, respectively. At this time only a light infection on grasses was found. Collections on grasses and grains near infected barberries in Kansas all proved to be *P. graminis secalis* (Pers.) Eriks. and Henn.

In Nebraska, uredinia on grasses and grains near barberries were first noted on June 10, 14, and 15, in Johnson, Merrick, and Polk counties and later in the season in Merrick, Hall, York, and Saunders counties. The

[1] Paper No. 119 of the Journal Series of the Nebraska Agricultural Experiment Station. Cooperative investigations between the Department of Plant Pathology, Nebraska Agricultural Experiment Station, and the Division of Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writer wishes to acknowledge his indebtedness to Dr. C. R. Ball, formerly of the Division of Cereal Crops and Diseases, to Mr. F. C. Meier, Division of Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture, and to Mr. H. H. Foster and Mr. Floyd Schroeder, former graduate assistants in the Department of Plant Pathology, Nebraska Agricultural Experiment Station.

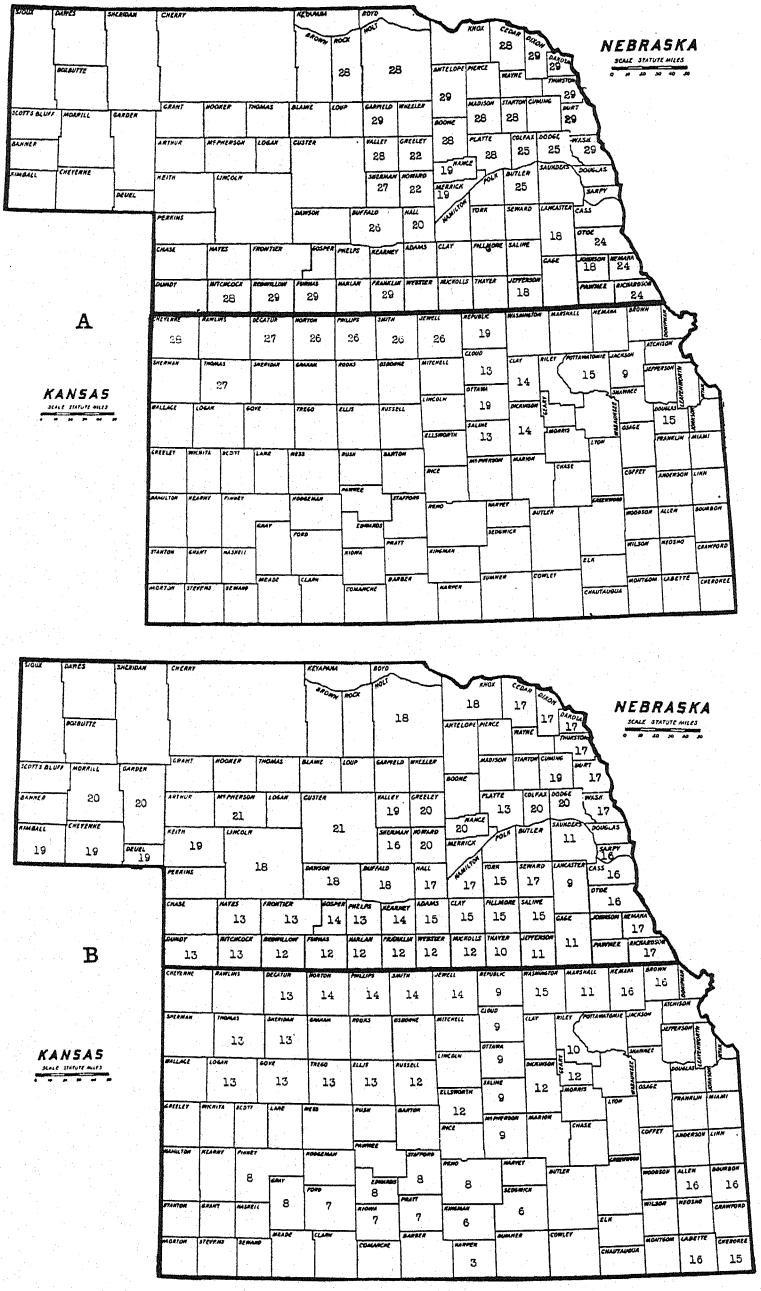


FIG. 1. Date in June when primary uredinia, not directly traceable to barberries, were collected in various counties in Kansas and Nebraska. A. 1928. B. 1929.

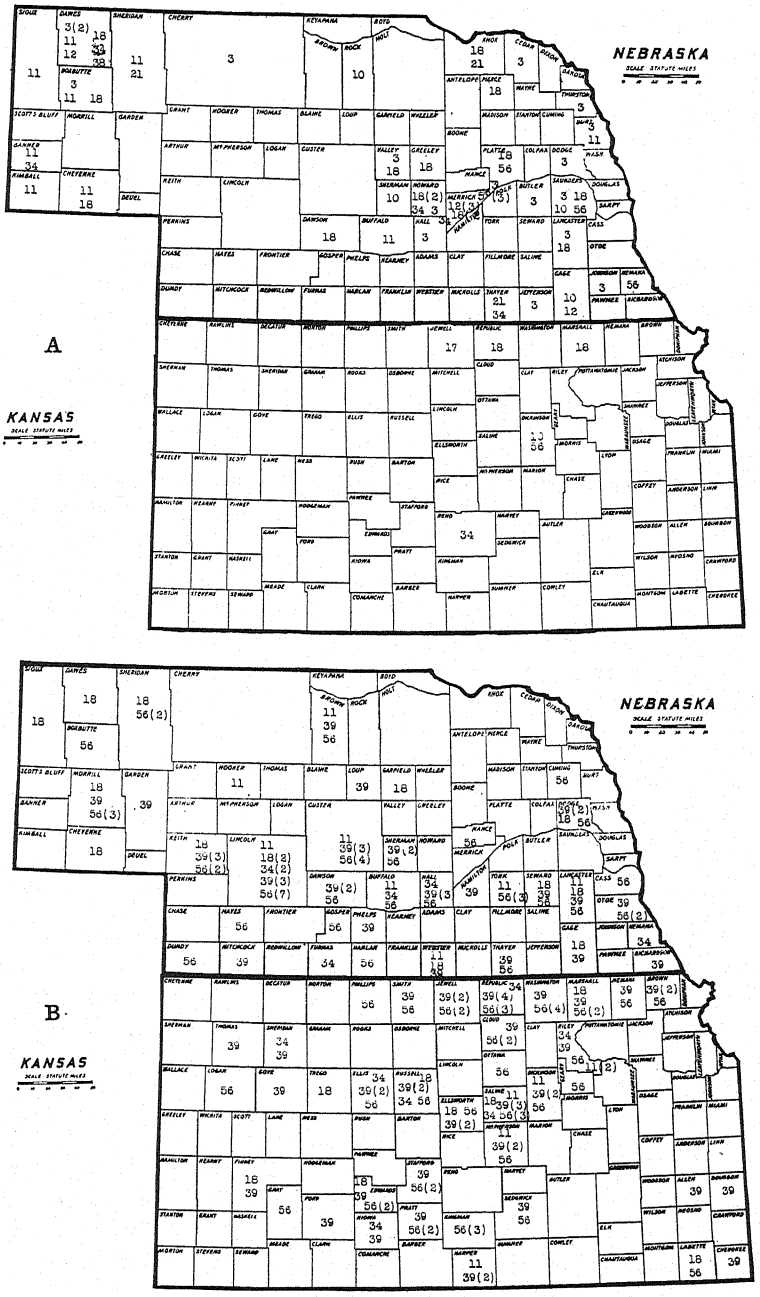


FIG. 2. Distribution of the physiologic forms of wheat stem rust collected in Kansas and Nebraska. A. 1928. B. 1929.

wheat stem-rust forms were identified from collections obtained near 5 barberry locations (Table 1). Physiologic form 3 was found in Johnson County (June 10), forms 12, 13, and 34, in Merrick County (June 28 to July 3), and form 56, in Saunders County (July 2). The uredinia found near infected bushes in Johnson County were the first to be discovered in Nebraska and antedate by 1 week the finding of uredinia not directly traceable to barberries.

Not Directly Traceable to Barberries. In northern Kansas, the first primary uredinia were found the second week in June. Primary uredinia were not observed in northwestern Kansas until the last week in June (Fig. 1, A). In Nebraska, primary uredinia were first observed on winter wheat June 18, in Jefferson, Johnson, and Lancaster counties. The following week a trace of stem rust could be found over most of the State. Stem rust developed very slowly and few secondary and succeeding generations of urediniospores were found. Owing to the fact that stem rust was late in making its appearance, wheat was harvested without any estimable loss from this rust.

Five physiologic forms were identified from the collections made in Kansas, while 9 forms were obtained from the collections made in 56 counties in Nebraska (Table 1 and Fig. 2, A). Of the 9 forms found in Nebraska only 2, forms 3 and 18, were collected throughout the entire season. Apparently these forms were the dominant and prevailing ones in Nebraska, as they were found in all parts of the State. Form 11 seemed to have been generally distributed over the wheat section of western Nebraska. The other physiologic forms were found in small amounts but fairly well scattered over eastern Nebraska. With the exception of form 17, all the others present in Kansas were found also in Nebraska.

PHYSIOLOGIC FORMS FOUND IN 1929

Near Barberries. In Kansas, a total of 11 properties with barberries present was observed in some detail for a period of several weeks in 5 counties. Notes made on several of these locations are presented in order to show the rate of development of rust on the near-by grains and grasses.

Riley County. Hedge with a trace to light infection. Aecia mature May 7. Occasional uredinia first noted on *Agropyron smithii* May 30. On June 8 a trace of infection was found on *A. smithii* 75 ft. and on *Hordeum pusillum* 20 ft. north of the hedge; whereas, 10 to 15 ft. from the hedge, a prevalence of 5 per cent infection was noted. About 10 ft. to the east of the hedge *A. smithii* had only a trace of rust. No rust was found to the west and south of the hedge. Aeciospores infected wheat and physiologic form 34 was later obtained. A collection of uredinia on *H. pusillum* proved to be the rye stem rust.

Saline County. Hedge with a trace of infection. Aecia mature May 9. Occasional uredinia noted on *Agropyron smithii* and *Hordeum pusil-*

TABLE 1.—*Annual occurrence of physiologic forms of Puccinia graminis tritici in Kansas and Nebraska*

Physiologic form No.	Number of times collected									
	Uredinia near barberry		Primary uredinia		Secondary and succeeding uredinia		Fall uredinia		Total	
	Kans.[a]	Nebr.[b]	Kans.	Nebr.	Kans.	Nebr.	Kans.	Nebr.	Kans.	Nebr.
1928										
3		1		4		8		4		17
10				2	1	2			1	4
11						8		1		9
12		2				3				5
17					1				1	
18		1		4	2	8		2	2	15
21						2		1		3
34		1		1	1	3			1	5
38						1				1
56		1		2	1	3			1	6
Total number of forms									5	9
Number of collections									6	65
1929										
11			5	3	1	5			6	8
18			7	7	1	7			8	14
34	1		6	4	1	2			8	6
39		3	41	24	1	8			42	35
56			35	29	6	11			41	40
Total number of forms									5	5
Number of collections									105	103
1930										
15			11	2					11	2
34			11			10			11	10
36	2		39	11		37			41	48
38			8	2		8			8	10
49			1			2			1	2
56				1		2				3
Total number of forms									5	6
Number of collections									72	75

[a] 1928. Collections from 5 properties near infected bushes in 3 northern counties were identified as *P. graminis secalis.*

1929. Collections from 11 properties near infected bushes in 5 northern counties in 8 instances were identified as *P. graminis secalis.*

1930. Collections from 7 properties near infected bushes in 2 northern counties in 5 instances were identified as *P. graminis secalis.*

[b] 1930. Collections from 2 properties near infected bushes in Greeley County were identified as *P. graminis secalis.*

lum May 29. On June 5 scattering infections were found 60 to 65 ft. west of the hedge on *A. smithii* and only 18 ft. on *H. pusillum*. About a 3 per cent prevalence was present on these 2 grasses within 10 ft. of the bushes. South of the hedge rust extended about 50 ft. on *A. smithii* with a 2 per cent prevalence within 10 ft. of the hedge. Very little change in the prevalence and severity of rust was noted on June 25. Collections from infected barberry leaves and grasses all yielded the rye stem rust.

Republic County. Hedge with a heavy infection. Aecia mature May 10. Occasional uredinia noted on *Agropyron smithii* and *Hordeum pusillum* on May 27. By June 4 a light infection had spread 25 ft. west of the hedge on *H. pusillum* and only 15 ft. on *A. smithii*. Within 10 ft. of the hedge a 25 per cent severity was found on both grasses, while farther away the percentage of infection dropped rapidly to less than 1 per cent. East of the hedge rust could be noted for 90 ft. on *H. pusillum* and only 25 ft. on *A. smithii*, while a 25 per cent infection occurred within 10 ft. of the hedge on *H. pusillum* and only 5 to 10 per cent on *A. smithii*. To the south rust extended about 50 ft. on both grasses. North of the hedge *H. jubatum* had 2 to 3 per cent infection 200 to 275 ft. away. Within 100 ft. 3 to 5 per cent infection occurred. Within 50 ft. of the bushes the percentage of infection in *H. pusillum* increased to 15 per cent, and within 25 ft. to 25 per cent. By June 15 the rust had spread 160 ft. to the west and east and 750 ft. to the north. The percentage of rust varied from 2 to 25 per cent near the bushes. No additional spread or increase in the severity of rust was noted after this date. Collection from infected barberry leaves and grasses all yielded the rye stem rust.

Smith County. Two bushes with a light infection. Aecia mature May 11. On May 31 an occasional pustule was found 15 to 20 ft. from the bushes on *Hordeum pusillum* and *H. jubatum*. By June 14 a 5 to 10 per cent infection was present on *H. pusillum* within a 10-ft. radius of the bushes. A trace of infection occurred on *H. pusillum* and *Hystrix patula* up to 60 ft. north of the bushes. *Hordeum jubatum* had a 50 to 60 per cent infection within 15 ft. of the bushes, while only a trace could be found on *Agropyron smithii*. Collections made from infected grasses all yielded the rye stem rust.

The above notes serve to give an idea of the development and rate of spread of stem rust from infected barberry bushes. At 8 such locations in Riley, Saline, Republic, and Smith counties rye stem rust was found near barberries, while wheat stem rust occurred only on 1 property with infected bushes. (Table 1). Uredinial collections on grasses from near 2 locations failed to infect wheat, oats, or rye.

In Nebraska, only 1 property with infected barberries was found rather late in June. Collections made on *Hordeum jubatum* near the bushes and from barley $\frac{1}{8}$ and $\frac{1}{4}$ mile away all yielded the wheat stem rust, which was later identified as physiologic form 39. (Table 1).

Not Directly Traceable to Barberries. The first primary uredinia were collected June 3 in Harper County, Kansas. A few days later primary uredinia were found in a number of the southern counties and were, for

the most part, fully developed and easy to find. In the western part of the State only a light primary infection occurred and rust was harder to find. In figure 1, B the date in June that primary uredinia were collected in the various counties is indicated.

The first uredinia not traceable to barberries were found in Lancaster County, Nebraska, June 9. During the same week a trace of rust was found across the whole southern part of the State. By the third week in June stem rust could be found in small amounts in all parts of Nebraska. Because of hot, dry winds, during the last 2 weeks in June, however, secondary infections developed slowly. While there was a fairly well-scattered primary infection throughout both States early enough to have produced a rust epidemic, the weather conditions were such that the wheat matured rapidly under the influence of the hot, dry winds, conditions extremely unfavorable for the further development of stem rust. Thus, there was no estimable loss from wheat stem rust in Kansas and Nebraska in 1929.

From the collections in Kansas representing primary uredinia, 5 physiologic forms were identified. (Table 1 and Fig. 2, B). The same 5 forms also were obtained from the collections of secondary and succeeding uredinia. It is of interest to note that all collections of infected barley yielded negative results when placed on wheat. It is apparent that forms 39 and 56 were the dominant and prevalent ones in Kansas. In many instances they were isolated from the same collection, so that they were closely associated throughout the State. The other 3 forms were found fairly well distributed but in smaller amount over most of Kansas.

The same 5 forms found in Kansas also were identified from the collections representing primary, secondary, and succeeding uredinia in Nebraska. The number of times the forms were found and their distribution are given in table 1 and figure 2, B. Here, again, it will be noted that forms 39 and 56 were the dominant and prevailing ones and were associated many times as mixtures in the same collection. The other 3 physiologic forms were fairly well distributed in smaller numbers over most of Nebraska.

A number of rust-infected wheat plants were sent the writer from points in Texas and Oklahoma, and form 39 was consistently isolated from these collections.

PHYSIOLOGIC FORMS FOUND IN 1930

Near Barberries. A total of 7 properties in 3 counties in northern Kansas, with barberries present, was observed the middle of June. Aecia were found on most of the bushes, with only a trace to a light infection, and from an occasional to few aecia per leaf. All but 2 collections of infected grasses near these bushes yielded rye stem rust. Two collections of wheat from near an infected barberry were identified as form 36 (Table 1). A rather heavy

infection occurred on winter wheat adjacent to these bushes on June 19, the severity varying from 25 to 50 per cent near the bushes to a trace to 25 per cent 70 ft. away.

Only 2 instances of spread of rust from infected barberries were located in Nebraska. On July 5, scouts found 2 properties in Greeley County, where bushes were moderately infected. All collections of grasses and grains near the bushes and for a mile away yielded rye stem rust.

Not Directly Traceable to Barberries. The first uredinia in Kansas were collected on May 29 in Harper County, but it was somewhat later before uredinia could be readily found. Between June 9 and 24 over 100 lots representing primary uredinia were collected in 54 counties of Kansas (Fig. 3, A). While stem rust was found in all sections of the State the second week of June, it was not a heavy primary infection. The wheat matured rapidly and the yields for the most part were high. No measurable loss from stem rust occurred in Kansas.

The first uredinia not directly traceable to barberries were found on June 8 in Jefferson and Thayer counties, Nebraska. During the same week primary uredinia were found across the whole southern half of the State. By the end of the third week in June primary uredinia occurred in small amounts in all parts of Nebraska (Fig. 3, A). Some secondary and succeeding generations of uredinia were collected, but, owing to the rapidity with which the grain matured, no estimable loss from stem rust occurred. Over 200 collections in 76 counties were obtained in the State in 1930, some of which were lost when the rust was transferred to wheat in the greenhouse.

Through the cooperation of workers in several Southern States, collections of wheat stem rust were obtained from Denton, San Antonio, Dalhart, Big Springs, and Canyon, Texas. From this material form 36 was consistently obtained. Likewise, material from Stillwater, Lawton, Woodward, and Norman, Oklahoma, yielded form 36, together with form 38. From a few collections made in Missouri and Arkansas form 38 was obtained.

Five physiologic forms were identified from the collections made in Kansas (Table 1). It is apparent from figure 3, B that form 36 was the dominant and prevalent one, present as primary uredinia in Kansas. Physiologic forms 15, 34, and 38, while found in smaller numbers, were fairly well distributed over the State. Only one collection of form 49 was found in southeast Kansas.

The same 5 physiologic forms found in Kansas also were present in Nebraska. A sixth form was identified from 3 lots of material widely scattered over the southern part of the State (Table 1). Again, it will be noted that form 36 was the prevailing and dominant form in Nebraska. Forms 34 and 38 were fairly abundant and widely scattered. Only an occasional collection of the other 2 physiologic forms was found.

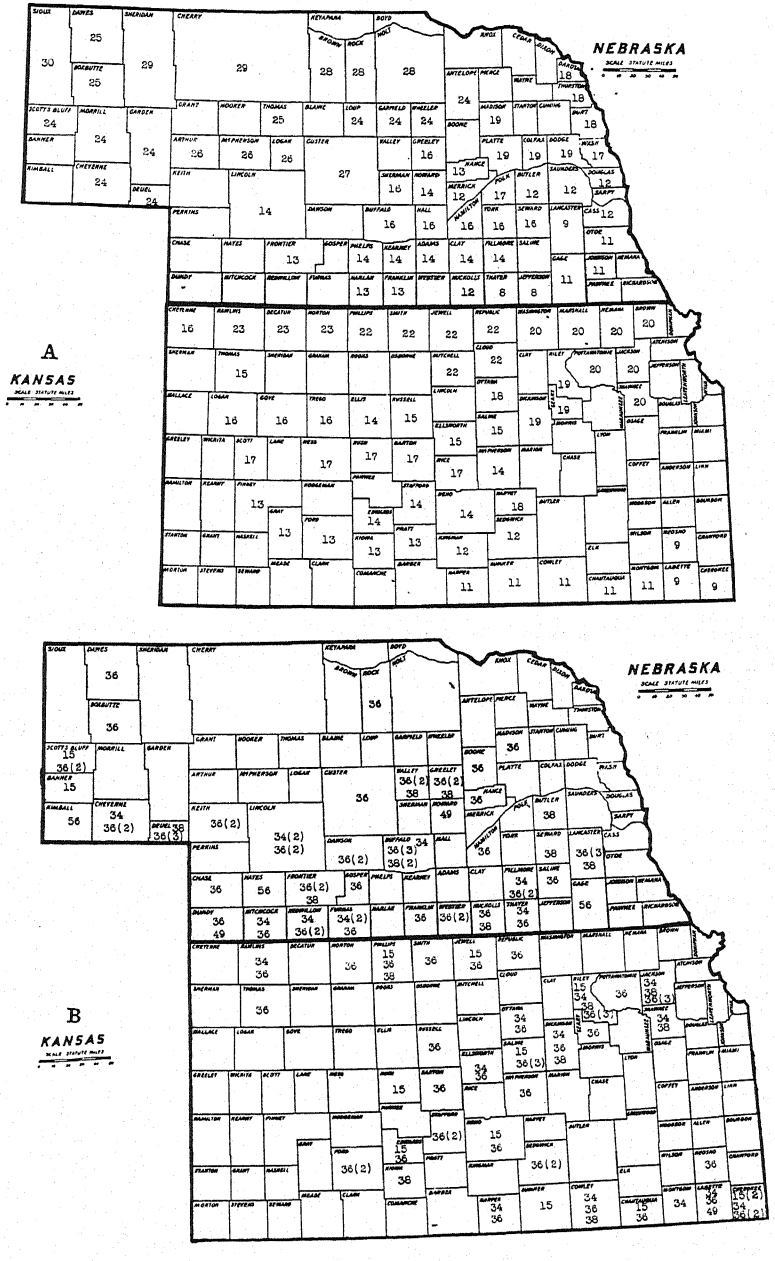


FIG. 3. A. Date in June, 1930, when primary uredinia, not directly traceable to barberries, were collected in various counties in Kansas and Nebraska. B. Distribution of physiologic forms of wheat stem rust collected in Kansas and Nebraska in 1930.

DISCUSSION

Of the 11 localities with infected barberry bushes present, under observation in Kansas since 1925 (3), in only 2 instances has wheat stem rust been found on near-by infected grasses and grains. In nearly every case the rye stem rust has been present. The same bushes visited over a 5-year period in Republic County have consistantly yielded rye stem rust.

With the continued eradication of barberries in Nebraska, it becomes more difficult to find infected barberries spreading rust early in the season. The spread of wheat stem rust from barberries is not so much a factor as it was in former years.

Primary uredinia not directly traceable to barberries appear usually in small amounts, but widely distributed in Kansas, about the first of June, and usually by the end of the same week in southern Nebraska. In occasional years, as in 1928, primary uredinia appear somewhat later. In both western Kansas and Nebraska uredinia are found from 1 to 2 weeks later.

The winter wheats commonly grown in Kansas are Black Hull, Kanred, and Turkey, and in Nebraska, Nebraska No. 60, Kanred, and Turkey. Black Hull, Turkey, and Nebraska No. 60 have proved susceptible to all the physiologic forms found in these 2 States. Kanred, however, is resistant to a number of the forms. It has been noted also that fields of beardless wheat, found occasionally in this area, usually are more heavily infected than adjoining fields of bearded wheat. No direct evidence is at hand indicating that the variety of winter wheat grown in this region influences the prevalence of any one physiologic form or combination of physiologic forms.

In table 2 are listed the physiologic forms found in Nebraska during 6 years. (3). In all, 20 physiologic forms have been identified. Of these 20 forms, a number have been found only once during 1 year, while a few have been collected in fairly large amounts during only 1 season. Four forms have been found during 2 seasons, 3 for 3 years, 2 for 4 years, and 2 forms have been found in 5 out of the 6 years studies have been in progress. In other words, there are common and uncommon forms. Physiologic forms 11, 18, 34, and 36 can be classed as common or prevalent forms, while forms 10, 21, and 56 have been present in 3 out of 6 years.

The simultaneous appearance of a physiologic form over a wide area in June indicates that barberries are not the primary source of inoculum. During some years the same form present over a wide area has also been found near infected barberries, but this form must have been present the preceding year, since viable telia must overwinter near-by in order to produce infection of the barberry. The uncommon forms for the most part are apparently forms localized near barberries, since they have not been found in Kansas or southward. The common or prevalent forms present in

TABLE 2.—*Annual occurrence of physiologic forms of Puccinia graminis tritici in Nebraska, 1924–1930*

Physiologic form No.	Number of times collected						
	1924	1925	1926	1928	1929	1930	Total
3	12			17			2
6	1						1
10	4		3[a]	4[a]			3
11	34		13[a]	9	8[a]		4
12	2			5			2
15						2[a]	1
17	3						1
18	13	8[a]	50[a]	15[a]	14[a]		5
21	5[a]	4[a]		3			3
24	2						1
25	1						1
29	2						1
31		5					1
32	3	1					2
34	9		7[a]	5[a]	6[a]	10[a]	5
36	11	20[a]	1			48[a]	4
38				1		10[a]	2
39					35[a]		1
49						2[a]	1
56				6[a]	40[a]	3	3
No. of forms ... 20	14	5	5	9	5	6	
No. of collections	102	38	74	65	103	75	

[a] Forms also found in Kansas and southward.

Data for 1924, 1925, 1926 obtained from Nebr. Agr. Exp. Sta. Res. Bul. 52.

Nebraska in any one year have in most instances been found also in Kansas, Oklahoma, and Texas (2).

Another striking relation is that the common or prevalent forms are rather akin to each other, varying in their reaction on 1 or 2 of the differential hosts. Thus, forms 3, 18, and 36 differ only in their reaction on Kubanka, forms 10 and 39 on Marquis, 21 and 34 on Kanred, and 11 and 15 where the type of infection on some of the differential hosts differs only in some minor degree.

It is of interest at this point to compare the physiologic forms found in Nebraska and southward with those identified by Stakman, Levine, and Wallace (5) and Wallace (6). A direct comparison for the years 1926

and 1928 can be made. In Nebraska forms 10, 11, 18, 34, and 36 were determined from 74 collections (Table 2). Stakman *et al.* from 8 collections identified 3 physiologic forms, namely, 18, 21, and 36. In Texas they found 7 forms present, 11, 17, 18, 19, 21, 36, and 39. From 4 collections sent from Texas, Peltier and Thiel (3) obtained forms 11 and 10, a form closely allied to 39.

Of the 7 forms determined by Stakman *et al.* in Texas, 5 were found in Nebraska by both groups of workers, if we assume that forms 10 and 39 are akin. Although they did not find form 11 in Nebraska, it was determined by us to be one of the most widely distributed forms in the State. The fact that we determined this form from Texas material and again in Nebraska a number of times, using the same methods of identification, obviates the attempt of Stakman, Levine, and Wallace (5) and Wallace (6) to assign form 11, as determined by us, to form 32.

In 1928 9 forms, 3, 10, 11, 12, 18, 21, 34, 38, and 56 were found in Nebraska. Stakman *et al.* identified 7 forms, 17, 21, 32, 36, 38, 39, and 49. Of the 8 forms found by them in Texas, 6 were identified from Nebraska. As in 1926, form 11 was found by Stakman *et al.* in Texas and Kansas but not in Nebraska, although it was identified from 9 collections by the writer.

Thus, while the physiologic forms found in Nebraska and southward were not always the same as identified by others, enough of the same forms were identified by both groups of workers to show that there is a movement of rust spores from the south into Nebraska.

Popp and Craigie (4) report that the first definite spore shower of the season occurred over Manitoba on June 17, 1929, and it was apparently correlated with a sluggish low-pressure area, responsible for a general air movement from the Mississippi Valley into Canada. At this time stem rust was present over all of Kansas and southward and over the greater part of Nebraska. A comparison of the physiologic forms found in Kansas and Nebraska with those later found in Canada should show some similarity if their contentions that spores present over Manitoba originated in this region.

In Kansas and Nebraska the 2 widely distributed forms were 39 and 56, while Newton and her associates (1) found 36 and 49 to be the prevailing forms in Canada. A number of collections of forms 21 and 38 also were made. From 240 collections made in the 3 Prairie Provinces of Alberta, Saskatchewan, and Manitoba they isolated a total of 16 forms.

Form 56, widely distributed in Kansas and Nebraska, was found by them in Canada, while form 39, identified 35 times in Nebraska, was found only once in Canada. On the other hand, form 49, widely distributed in Canada, was not present in Nebraska. Form 36, also found a number of

times in Canada, was not present in Nebraska, unless we consider forms 18 and 36 to be identical, as suggested by Wallace (6). Only 3 forms present in Nebraska and Kanasas in June were later found in Canada, so that the evidence of a spore shower occurring over Manitoba originated from spores in the winter-wheat belt is not conclusive, if it is based on the prevalence of the physiologic forms determined in the 2 regions.

The evidence presented suggests that there is each year a movement of urediniospores from local overwintering centers in the south into Kansas and Nebraska. The amount and distribution of inoculum vary with the prevailing environmental factors each season. This movement can not as yet be traced with certainty, since inoculum also originates from infected barberries yet to be eradicated in these and other States in the winter- and spring-wheat areas of the Mississippi Valley.

SUMMARY

Intensive studies of the primary sources of wheat stem rust in Kansas and Nebraska, based on observations of the time of its appearance, amount, and distribution and on the annual occurrence of physiologic forms of *Puccinia graminis tritici*, were made in 1928, 1929, and 1930.

In the majority of instances infected barberry bushes in northern Kansas were observed spreading *P. graminis secalis* to near-by grains and susceptible grasses. In Nebraska only 1 infected bush was found spreading *P. graminis tritici* prior to the appearance of primary uredinia not directly traceable to barberries.

Primary uredinia not traceable to barberries were found during the first week in June in Kansas and by the second week in southern Nebraska in 1929 and 1930. They appeared somewhat later in 1928.

No estimable loss from wheat stem rust occurred in either State in any of the 3 years.

The physiologic forms identified in Nebraska were found also in Kansas and southward in most instances.

The evidence presented suggests that there is a movement of urediniospores from local overwintering centers in the south, into Kansas and Nebraska each season. The time of the appearance, amount, and regional distribution of this initial inoculum varies with the prevailing environmental conditions.

The exact amount and distribution of wind-blown urediniospores reaching Kansas and Nebraska cannot be definitely determined, since inoculum also originates from infected barberries not yet eradicated in these and neighboring States.

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THE TECHNIQUE OF ISOLATION IN MICROBIOLOGY

SYDNEY DICKINSON[1]

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INTRODUCTION

For a number of years the value of reliable methods of single-cell isolation has been recognized in all branches of microbiology, but only recently has the essential need for accurate methods of single, particular cell isolation[2] become apparent. There is, however, no one method satisfactory for all types of material; consequently, it is proposed to describe a number of methods, some of which are new, which will embrace all types of material, including bacteria and algae. It is proposed neither to describe methods, such as the double-plate method of isolating the sporidia of *Tilletia tritici* (Bjerk.) Wint. (4), which rely on the self ejection of the spore from its hymenium, nor to describe methods dependent on the growth-differentiating properties of chemical substances. Such methods as these are not, in general, sufficiently reliable for single, particular cell isolation. All the methods to be described have a physical background and have been used and tested by the writer.

These methods may be arranged according to the means employed in separating the cells, the one from the other, *viz.*:

I. Cells separated by chance, usually in a volume of liquid, *e.g.*, the dilution and pipette methods, etc.
II. Cells separated by design when lying in one plane, *e.g.*, the dry-needle and Dickinson methods, etc.
III. Cells separated by force, *e.g.*, the microscissors method, etc.

While the methods included in the first 2 sections are applicable to almost all types of material in which cells are not united, the dry-needle method is suitable only for relatively large, thick-wall cells, and the Dickinson method is best suited for rather smaller cells. On the other hand, the methods included in the third section are adapted for dealing with all types of material whose cells are fastened together in filaments, plates, or masses.

It is hoped to describe methods for separating nuclei of different kinds in coenocytic cells at a later date.

I. CELLS SEPARATED BY CHANCE USUALLY IN A VOLUME OF LIQUID

The original means of isolation were the "dilution" or "poured-plate" methods. While there are innumerable variations, these methods depend,

[1] Rockefeller Foundation Fellow at the University of Minnesota, on leave from the University of Wales.

[2] By single particular cell isolation is meant the isolation by choice of single cells distinguished either by reason of their morphology or position of origin, etc.

in principle, on the making of an even suspension of cells of such a concentration that on an average one drop of the suspension will contain only one cell. This suspension is either made in agar before gelation and then poured in a thin layer in a Petri dish, or it is made in a sterile liquid. In the latter case drops of the suspension are put at marked places on the surface of nutrient agar in a Petri dish. Incubation follows, and, as soon as possible after germination, the plates are examined under a microscope. Colonies that have arisen—as far as can be ascertained—from one cell and that are well separated from other colonies are then cut out from the agar and transferred to stock-culture tubes.

In the case of bacteria, owing to the advisability of determining at once, by direct observation, the presence of an isolated cell, films are made either of agar or of gelatin in various ways on cover slips, etc., or, as in the modified Burri's method (22) on celluloid strips with Indian ink. One drop of a suitable dilute suspension is placed on the agar film or mixed with the Indian ink, and a microscopic examination is made at once to determine whether the isolation of a cell has been achieved. Topley, Barnard, and Wilson (28), after covering the isolated bacterium with a mercury droplet, exposed the film to ultra-violet light and so killed the rest of the bacteria.

To help recognition of the microscope field during and after incubation, Ørskov (21) scratched his cover slips with a diamond, and Gardner (13) used ash prepared by burning a loopful of serum. To facilitate the removal of agar blocks containing isolated cells or colonies, Keitt (18) has devised two instruments, while Ezekiel (12) has suggested modifications of his method. One of these tools is designed for the cutting out of cylinders of agar and the other for lifting these cylinders out of the surrounding agar and transferring them to the stock culture tubes. The instrument used by Sass (25) served for both these purposes. La Rue (19) has invented a "cutting out" device which is screwed into the nose-piece of a microscope. This enables the agar cylinders to be quickly and easily delimited. With bacteria, Ørskov (21) used a "harpoon" method for subculturing the growth of single-cell colonies from his agar films, while Gardner (13) improved this method by arranging for the whole process of subculturing to be carried out under microscopic observation. A review of the four most practical and widely used methods in bacteriology has recently been made by Stoughton (27).

The chief objections to the dilution method and its variations are: firstly, that there is no absolute guarantee of the origin of a colony from a single cell, unless the growth has been continuously observed under the microscope; secondly, that single cells only are isolated; and lastly, that a considerable amount of time and labor is consumed.

The first step in the direction of greater reliability was the "Barber pipette" method (2). Originally this was devised for use with bacteria, but it has been applied with success to fungi, algae, etc. Described first in 1904, in principle it consisted in the taking up of a single cell (or bacterium) from a drop of liquid on a cover slip inverted over a modified van Tieghem cell into a micropipette under the microscope. The end of the pipette containing the drop of liquid was broken off and dropped into a flask or tube containing a sterile nutrient medium. The chief difficulty in this method is the cleansing and greasing of the cover glasses, so as to allow small enough water drops to be formed. A recent and full description of this method has been written by Gee and Hunt (14). While it is reliable, though slow and difficult, for bacteria, it can not be used to any great extent where single particular cells are to be isolated. Edgerton (11) has modified the Barber pipette method in using a closed capillary, and so, isolated single ascospores or asci of *Glomerella cingulata*. The spores were sucked into the capillary and ejected by the application of ether and heat, respectively, to the closed end of the pipette. Recently, Craigie (7) has described a method of catching the sporidia of rust fungi, as they are produced, by means of a pipette for this purpose. Ashworth (1), likewise, has used a pipette method for the same type of work.

Schouten (26), somewhat later than Barber, described a method that requires as the vehicle of isolation the film of liquid formed across a loop of very small dimensions. Owing to the difficulties of making such a loop, the method has not been adopted extensively by bacteriologists, but an extension of the method, using larger loops, has been used with success by Dunn, working with *Sclerotinia* spp. (10).

II. CELLS SEPARATED BY DESIGN WHEN LYING IN ONE PLANE

Now it will be appreciated that the isolation of the separate cells by the methods previously mentioned has depended upon use of a liquid in which the cells are separated in a haphazard manner.

When the cells to be isolated are relatively large and dry, *e.g.*, spores, the most satisfactory method is that known as the dry-needle method, described by Hanna (15). This method depends on the fact that a spore will adhere to the point of a dry needle, although (Hanna used a steel needle) the reason for adhesion is not clearly understood. Hanna showed that it was not the ordinary electrical phenomenon of the attraction of oppositely charged particles. It may be that spores, unless specially dried, have a surface film of water (or mucilage) and that the adhesion is due to surface tension. In the writer's experience, absolutely dry spores will not adhere to a dry needle in a dry atmosphere. They will adhere, however, if there is a very small drop of water at the needle tip.

In practice the material—in Hanna's case, basidiospores from a gill of Coprinus—is allowed to fall or is dusted onto a microscope slide. The needle point is brought up to a suitable spore by hand under the microscope. The spore adheres to the needle, and it is then rubbed onto the surface of the agar in a test tube. The lower limits of size of spores for this method are about 20–30 μ. (Fig. 1, A.)

For wet material or for very dry spores, it has been found best to place a small mass of the material on a microscope slide and to add a drop of sterile water. Then, using a low-power microscope and two mounted steel

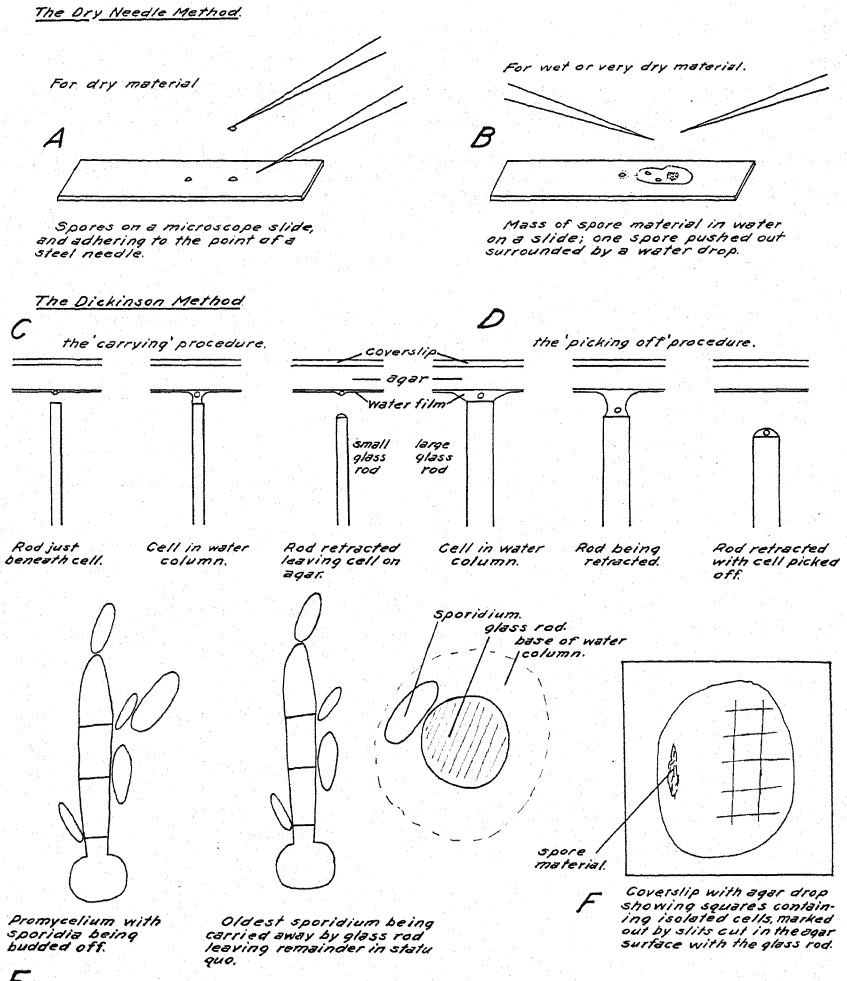


FIG. 1. Dry-needle and Dickinson methods of isolation.

needles, a cell is separated from the mass, and pushed out of the drop of water along the surface of the microscope slide. A small drop of water will be carried with the cell. This drop of water is allowed to evaporate almost entirely, and the cell may then be picked off by a dry needle point. This method can be used only under a low-power microscope and requires a certain delicacy of touch in handling the needles. (Fig. 1, B.)

For small cells, from 30–20 μ down to the limits of microscopic vision, the most satisfactory method appears to be the Dickinson method (8, 9).

In practice, the method consists in "carrying" the cells by means of a fine glass rod from their original position on the surface of an agar drop on a cover slip to the opposite side of the agar drop. The new positions are marked by slits made in the agar surface with the glass rod. (Fig. 1, F.) The pieces of agar containing the isolated cells are cut out and then trans-

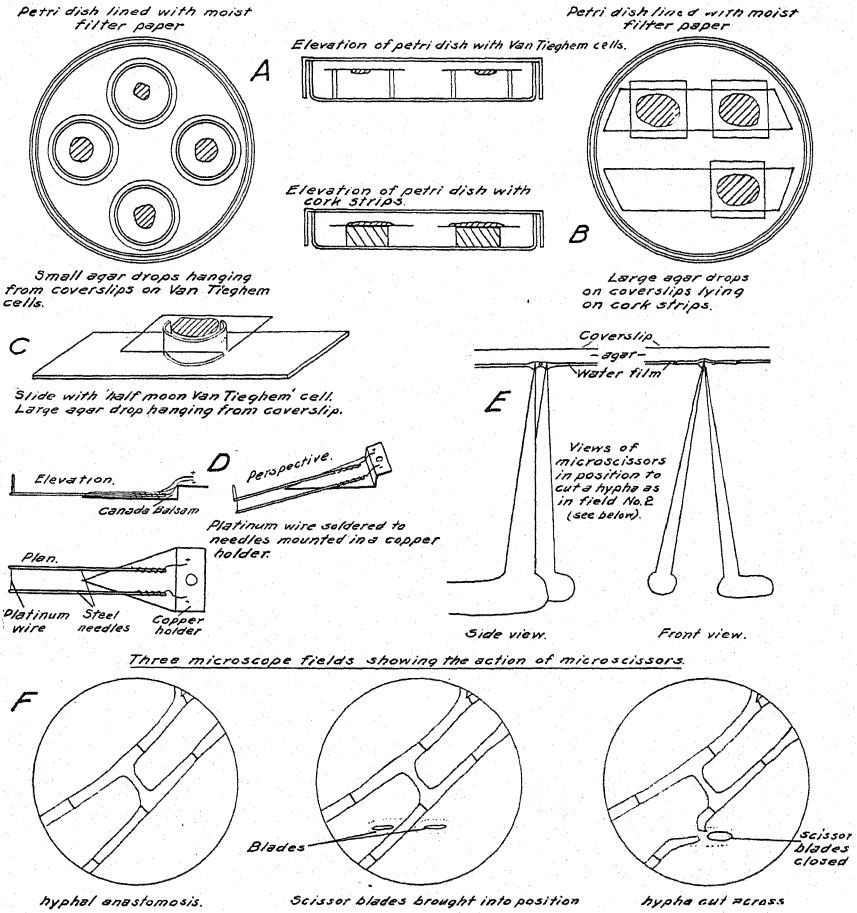


FIG. 2. Separation of cells by force.

ferred to stock culture tubes with a sterile scalpel. The movement of the fine glass rod is controlled by some mechanical device (see later), while the cover slip is inverted over a crescent-shape van Tieghem cell fastened to a microscope slide (Fig. 2, C), and this is moved by hand or by a mechanical stage.

This method is suitable for the separation and isolation of particular cells in a growing culture because it allows the separation of a particular cell without altering the relative position of any of the neighboring cells, no matter how close they may be to the required cell. (Fig. 1, E.) That this method is speedy is shown by the fact that it is comparatively easy to isolate between 100 and 200 single cells (average length of longer axis 10 μ) in 8 hours.

The principle involved in this method is the utilization of the column of water that can be formed between a glass rod and the film of water on the surface of agar. The reason that a cell can be carried along in such a column of water is that the direction of the applied force, *i.e.*, the edge of the water column, is nearly parallel to the place of motion. When the column of water is very short, the direction of the applied force is at an angle to the place of motion. In such a case the surface tension of the water column surface is not sufficient to overcome the friction between the cell and the agar, and, in consequence, the cell is left behind. (Fig. 1, C, and D.)

Theoretically, a cell can not be picked off the agar by the glass rod unless the diameter of the rod is rather more than 3 times the longer axis of the cell. This has been verified by experiment. What happens when a cell is picked off onto a glass rod is that, with a rod of sufficient diameter, there is room for the cell to sink (by gravity) to the top of the rod. When the column of water is lengthened by the withdrawal of the glass rod, the cell is not drawn back onto the agar surface. In the case of a glass rod of insufficient diameter, while the cell may sink to the top of the glass rod at first, when the column of water is lengthened by the withdrawal of the rod, the cell is drawn back onto the agar surface, as there is not enough room for it on the top of the rod. It should be realized that the glass rod takes off only a water drop of a maximum volume equal to a hemisphere whose diameter is equal to that of the rod itself. (Fig. 1, C and D.)

Hanna (16) has amplified the original description of this method and has advocated, by using a glass rod of sufficient diameter, the picking off of the cells from the original agar drop on the surface of a cover slip and transferring them to a fresh agar drop. For such picking off of the cells, it is best to use a glass rod whose diameter is at least 5 times that of the longer axis of the cell. The writer uses the carrying of the cell in the column of water to another part of the agar for thin-wall cells (Fig. 1, C) and the

picking-off procedure for thick-wall cells. (Fig. 1, D.) The better procedure is, in general, the carrying rather than the picking off of the cells, because there is usually an appreciable number of cases of damage to the cells when they are picked off. It is thought that this is largely due to the evaporation of the surrounding water drop. As regards speed and ease of use, there is very little advantage in either procedure.

In order to be able to pick off cells easily, there must have been very little evaporation from the agar surface. To guard against this, Hanna advocated the use of small drops of agar on the surface of cover slips kept, when not in use, inverted on van Tieghem cells in Petri dishes lined with moist filter paper. (Fig. 2, A.) In the carrying procedure, the writer usually employs larger agar drops (about 2 cm. in diameter) and keeps them under less humid conditions on cork strips in Petri dishes lined with damp filter paper. (Fig. 2, B.)

It is not usually possible to isolate more than about 30 cells on an agar drop when using the carrying procedure. But where a greater number is required, the writer, after transferring the agar pieces containing the already isolated cells to stock-culture tubes, adds fresh agar to the cover slip and proceeds to carry more cells onto this new agar, etc. The only care, besides precautions against contamination, is to see that the surfaces of the old and fresh agar are level. It is as easy to pass from old to fresh agar as it is to pass from one part of an agar surface to another. It is not, however, in general, easy to pass over any place where the agar surface has been cut.

III. CELLS SEPARATED BY FORCE

The isolation of parts of filaments or masses or of any particular cell from a hypha or from cell tissue is complicated, as Harder found (17), by the need for separation as well as for isolation. As regards the separation or cutting out of a cell from a hypha, this is easily done by using a platinum wire (diameter 0.0003 in.) heated by a weak electric current (Fig. 2, D, for method of mounting wire). This cannot, in general, be advised because of the danger of damaging the wanted cell by heat (*cf.* Barnes, 3), but it has been used with success in separating the clamp-connection cells in *Coprinus* spp. Another method of cutting out single cells of hyphae is by means of a small piece of a razor blade, suitably mounted in a micromanipulator or isolator. The film of agar on which the hypha rests must be very thin, as there must be a hard surface against which the razor blade can press. As soon as a thin film of this sort is used, there comes the difficulty of guarding against the drying out of the film, as well as the preserving of the adjacent hyphae in *statu quo*. Lindegren (20) used a tool in the form of a Dutch hoe, made from chromel wire, for the cutting out of single ascospores from the asci of Neurospora. He was able to cut the

ascus wall with this tool because his material was dried and was lying directly on a glass cover slip.

For the isolation of parts of filaments, Brown's "hyphal-tip" method (5) is commonly employed. This, in essentials, is a modification of Edgerton's pipette method. It consists in the cutting out, by means of a fairly fine glass tube, of a cylinder of agar containing one hyphal tip under the microscope and then the blowing out of the agar from the glass tube into a stock-culture tube. This method, provided the hyphae are spaced sufficiently far apart and provided not too small a diameter tube be used, is both quick and easy. It is, however, limited in scope to the tips of growing hyphae. Harder (17) was successful in separating the clamp cells and basal cells in Pholiota. He killed one or the other of the component cells of the developing clamp connection by piercing them with a fine glass rod. He then isolated this cell by dragging away all the other filaments in its neighborhood. This procedure is not generally applicable and requires a high degree of skill.

The method devised by the writer is based on a suggestion made by E. C. Stakman and consists in cutting the hyphae in two with a pair of microscissors attached to a micromanipulator or isolator. The blades of these scissors are made of small pieces of an ordinary razor blade. They are mounted at the ends of circular steel rods. One of the blades moves by a rack-and-pinion movement past the other blade, parallel to the microscope stage. The other blade can be adjusted as to its height above the microscope stage, as well as its angle to the perpendicular. The result is that the blades can be adjusted so that their tips are on the same level and that these tips come in contact only when the movable blade passes the stationary one. (Fig. 2, E.) In addition, the pressure between the tips of the blades, on contact, can be adjusted.

The above-described adjustments are important because there is always a tendency for hyphae to slip between the blades or between one blade and the agar, rather than to be cut in two. Again, the tips of the blades have to be ground to the finest possible point, or about 5–10 μ in diameter. In general, it is possible only to cut hyphae growing on the surface of agar, but they can be cut with accuracy and without disturbing them, at all, provided the tips of the blades be ground to a really fine point. It must be emphasized, however, that the adjustment of the blades relative to one another is most important.

After the microscissors have been used for some time, it becomes necessary to clean the tips of the blades from adherent bits of agar. This is most easily done by regrinding the points. A couple of strokes along the oilstone with each surface is usually sufficient for the purpose.

In practice, the material is placed or is grown on agar drops on coverslips similar to those used in the isolation of small separate cells (Section II). After deciding on an individual hyphal cell, or, for that matter, a cell formed by the anastomosis of 2 adjacent hyphae, it is perfectly easy to separate this wanted cell by cutting the cells on either side of it. (Fig. 2, F.) Having completed the separation of the required cell, it is necessary to isolate it. This can be done with cells up to about 20 μ long with a glass rod, using the Dickinson method of isolation. Care, however, is necessary, because the cell ought not to be bent at all. While no case of mechanical damage has yet been proved to have occurred, the possibility must always be kept in mind. It seems best to use the carrying rather than the picking-off procedure because, in the latter, there is, first, the danger of drying out, and second, the cell walls at each end of the required cell are liable to stick to the glass rod, thus making it difficult to free the cell from the glass rod. While the transport of single hyphal cells is comparatively easy, when they are only about 20 μ in length, it becomes a matter of considerable difficulty in *Coprinus* spp., for example, where the cells are much longer. In such a case a satisfactory method seems to be to grip a piece of dead adjacent cell between the scissor blades and drag the cell along the surface of the agar until it is on a fresh part of it.

Micromanipulators,[3] *etc.* Numerous machines hvae been used or described by various workers (6, 16, 23, 24, etc.) for handling the pipettes, needles, glass rods, microscissors, etc., but it is not proposed to enter into a description of these various machines nor comment on their suitability. It must be pointed out, however, that there is no necessity to use a micromanipulator or isolator, except where material of small size is involved. The essentials required of a machine are to-and-fro and back-and-forward movements, in order to center the particular tool, and a fine vertical movement. The last is the most important of the three.

The writer has used and taught to students single-cell isolation with the following device, which requires no special apparatus at all. The material from which spores are to be isolated is placed on an agar drop on a cover slip, which is then inverted over a hole (Fig. 3, A and B) cut in a microscope slide. This slide is placed on the mechanical stage of the microscope with the hole—about $\frac{5}{8}$ inches diameter—directly over the condenser. (Fig. 3, D.) It is possible with delicate handling to manage without a mechanical stage. A cover slip is put on the top of the condenser and bears an L-shape glass rod, the vertical arm of which tapers to a fine point and is fastened to the cover slip by Plasticene. (Fig. 3, C and D.) The tapered tip of the glass rod is centered by hand, or may be centered by the

[3] The machine used by the author is manufactured and marketed by Messrs. C. Baker & Co., 244 High Holborn, London, England.

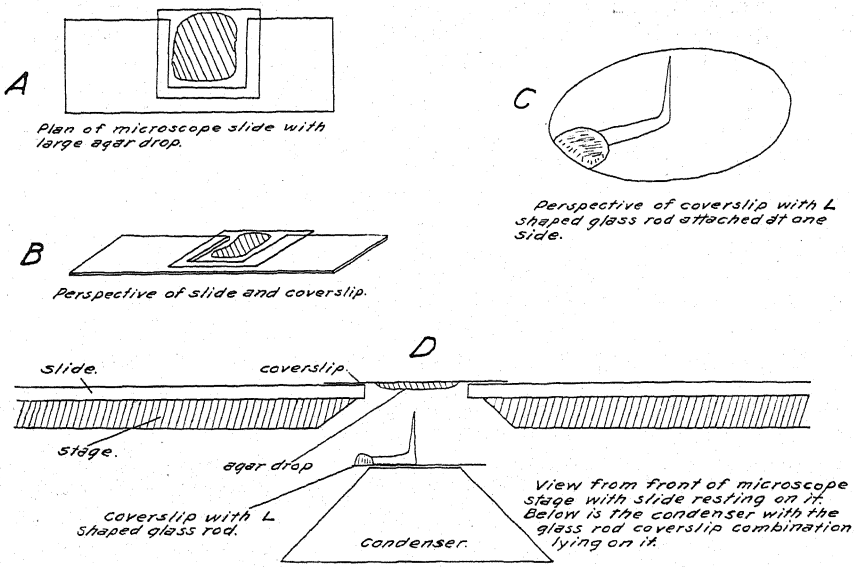


FIG. 3. How to obviate the use of a micromanipulator.

condenser centering screws, if they be present, and is then raised or lowered by means of the condenser rack-and-pinion movement. The actual handling of the spores, etc., is done by using either the dry-needle or the Dickinson method.

SUMMARY

A number of methods for the isolation of single cells have been mentioned and the principles underlying them compared. Those methods that allow the isolation of single particular cells have been described in detail. It is pointed out that for such isolation of medium and large-size separate cells, no special apparatus, other than a microscope, is required.

UNIVERSITY OF WALES,
CARDIFF, WALES.

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GIBBERELLA MONILIFORMIS ON CORN

R. K. VOORHEES[1]

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INTRODUCTION

Perithecia of *Gibberella moniliformis* (Sheld.) Wineland are produced on corn-leaf sheaths under natural conditions in Florida. The imperfect stage of this fungus, commonly referred to as *Fusarium moniliforme* Sheld., is a parasite on corn throughout the world (1, 2, 3, 7). In the southern part of the United States, where the climate is warm and humid during the latter part of the corn-growing season, the disease caused by this fungus is very destructive. In Florida the annual reduction in yield caused by *G. moniliformis* is estimated at 5 per cent of the corn crop.

This report deals with a description of the symptoms of the above-mentioned disease, as it was observed in the favorable environment of Florida, and studies pertaining to the characteristics of the causal organism, its pathogenicity, and the natural occurrence and artificial production of its perfect stage.

SYMPTOMS

In Florida, after corn plants have matured and the leaves begin to dry, the leaf sheaths and culms of many plants become covered with the pink spore masses of the Fusarium stage of the fungus, and the growth of the organism in the stalks sometimes so weakens them at the lower nodes that they are easily broken by the wind. Ear worms, which tunnel through the husks and shanks or enter the ears at exposed tips, open a pathway for the parasite to enter, resulting in moldy kernels adjacent to those damaged by the insects. Sometimes, all of the kernels of a diseased ear are covered with mycelium of the fungus, but usually the infected ears show no external signs of the disease and it is detected only when the kernels are tested in a germinator (Fig. 1, A). The damaged kernels either fail to germinate or produce weak seedlings that become covered with pink spore masses of the fungus.

The disease was observed in seedlings grown in sterilized soil inoculated with the parasite. The tops of infected seedlings turned yellow and wilted and the roots and mesocotyls became brown and shrunken (Fig. 1, B). Infection resulted either in the death or a stunting of the growth of the plant.

[1] Grateful acknowledgment is made to Dr. A. H. Eddins for his criticisms and suggestions.

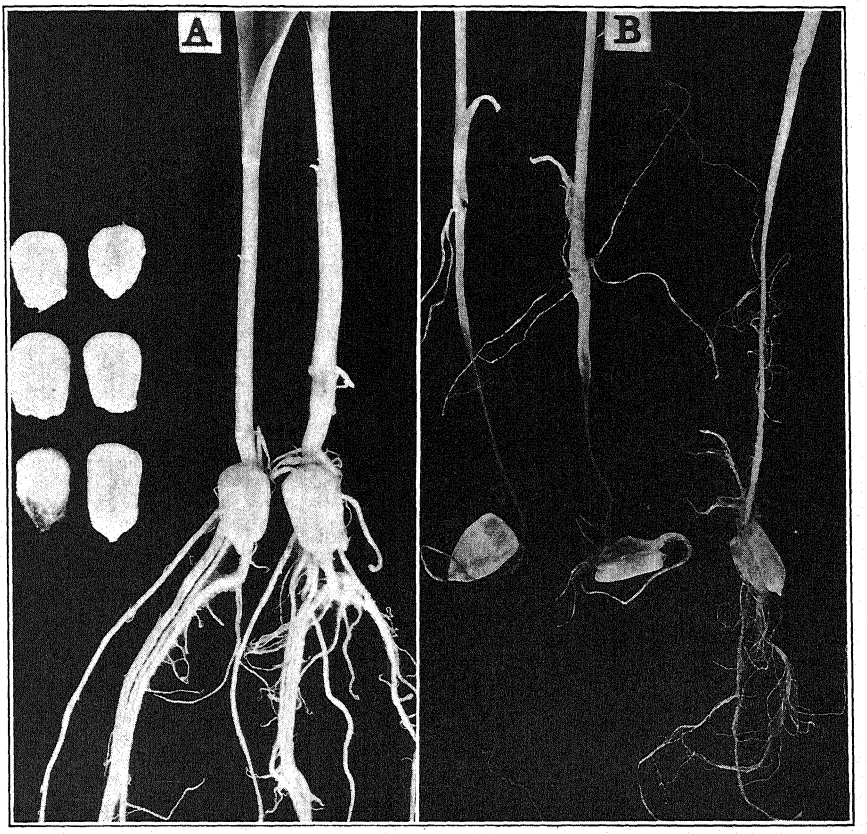


FIG. 1. A. Contrast between kernels naturally infected with *Gibberella moniliformis* (left) and disease-free kernels after 7 days' exposure in a rag-doll germinator. B. Seedlings grown in sterilized soil inoculated with *G. moniliformis* (left), and a seedling grown in sterilized noninoculated soil.

CAUSAL ORGANISM

Morphology

The morphological characteristics of the imperfect and perfect stages of *Gibberella moniliformis* growing on ears and stalks of corn and on potato-dextrose agar in pure culture were studied. With a few exceptions these characteristics were similar to and, in some cases, identical with those described by Sheldon (6) and Wineland (8). The main differences found are pointed out below.

Mycelium. Aerial and submerged mycelium ranged from scanty to medium dense and from dirty white to deep rose pink and pale hortense violet (4). The hyphae were hyaline, septate, and branched. On the host

the mycelium and spores of the fungus appeared as a white to pink felty mass.

Conidia. The simple and branched conidiophores formed at the tips and lateral branches of hyphae bore the microconidia either singly in long chains, or in heads or clusters. Microconidia from corn-leaf sheaths measured 6–15 × 2.5–5 μ, averaging 10.5 × 4.2 μ, and those formed on potato-dextrose agar in culture measured 4.5–12 × 2.5–4.5 μ, the average being 8.9 × 3.4 μ. The spores germinated within 12 to 24 hours in tap water at room temperature, and during germination they swell, usually become 1 to 2-septate, and send out a germ tube from each or only one of the cells.

The macroconidia are borne on aerial mycelium, in pseudopionotes or sporodochia. The spores are somewhat curved, especially near the apex, slightly constricted at the septa, and are 3 to 5-septate, the 3-septate spores predominating. Mature macroconidia from host material measured 26–55 × 3–5 μ, averaging 41.1 × 4.3 μ, and those from potato-dextrose agar measured 24–40 × 3–5 μ, the average being 31.5 × 3.7 μ. Upon germination the spores

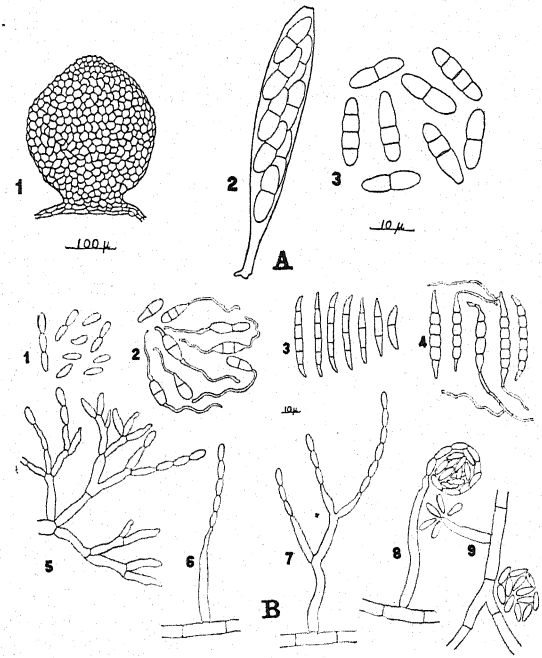


FIG. 2. Camera-lucida drawings of fruiting structures of the perfect and imperfect stages of *Gibberella moniliformis*. A. Typical perithecium, ascus, and ascospores produced on cornstalks. B. Microconidia and macroconidia produced on the host plant and potato-dextrose agar: 1, Typical microconidia; 2, germinating microconidia; 3, typical macroconidia; 4, germinating macroconidia; 5–7, various types of conidiophores; 8–9, clusters of microconidia at tips of conidiophores and along the mycelium.

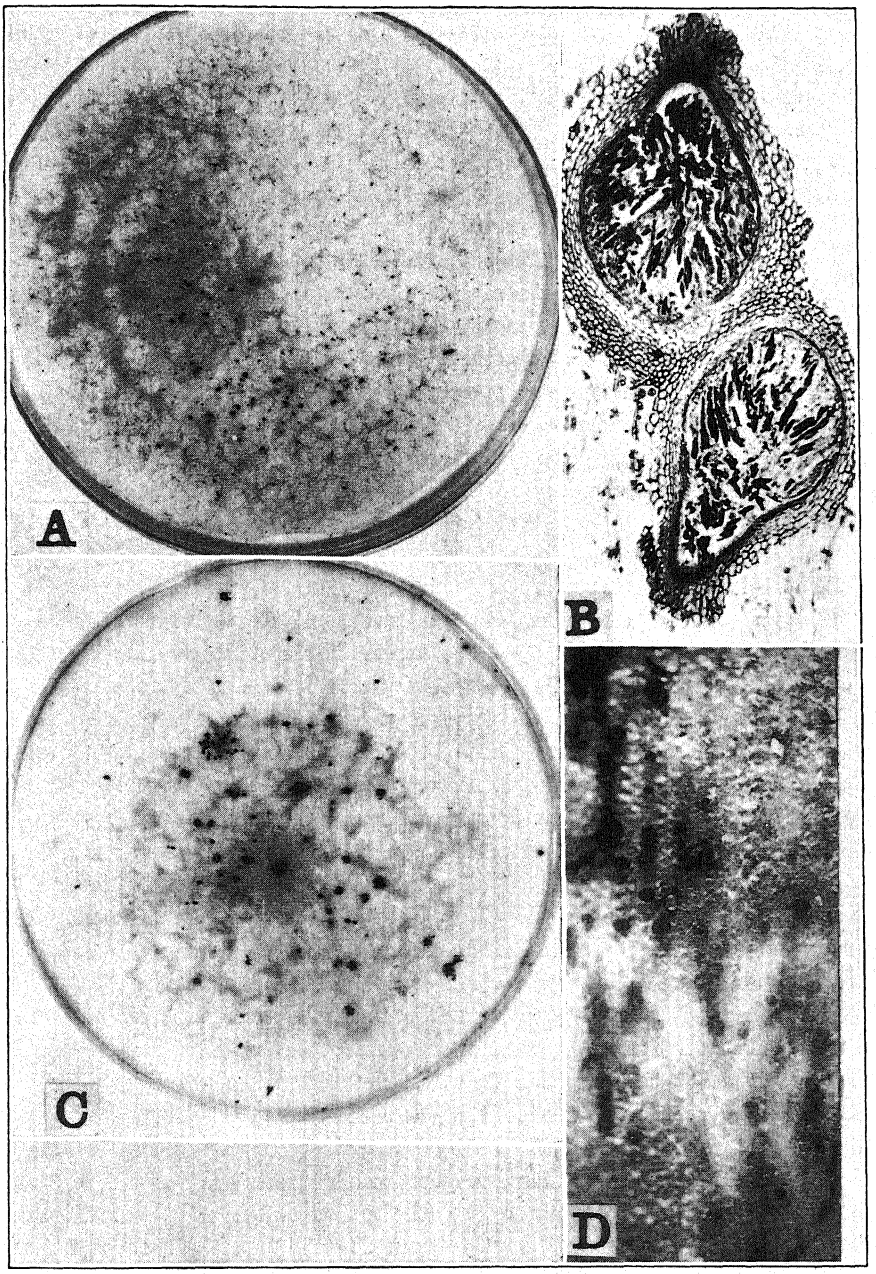


FIG. 3. A–C. Single-ascus cultures of *Gibberella moniliformis* on potato-dextrose agar. Black dots are perithecia. A. Top view. B. Photomicrograph of longitudinal section of perithecia. × 200. C. Bottom view. D. Photomicrograph of perithecia on cornstalk. × 10.

swell and usually become more septate and more constricted at the septa. The germ tubes may form from one or both ends of a spore and become septate and branched (Fig. 2, B).

Perithecia. Perithecia produced in pure culture on potato-dextrose agar were scattered or gregarious, free on the surface of the medium or embedded in the mycelium, and measured 250–300 × 300–350 μ (Fig. 3). Perithecia on cornstalks were scattered or gregarious, erumpent or free on the surface, and measured 210–300 × 275–380 μ (Fig. 3, D).

Ascospores. Each ascus contained 8 ascospores arranged irregularly in 2 rows. The spores were almost straight, rounded at the ends, and constricted at the septa, 1–3 septate, the 1-septate predominating (Fig. 3, B). Ascospores from host material and culture media were practically the same size, as those from corn leaf sheaths measured 12–20 × 4–6 μ, averaging 15.3 × 5 μ, while those from potato-dextrose agar measured 14–20 × 4–5 μ, averaging 17.5 × 4.7 μ (Fig. 2, A).

Taxonomy

The color of the substratum, formation of microconidia in chains, predominance of 3-septate macroconidia, and sizes of the 2 conidial spore forms of the imperfect stage of the causal fungus identified as *Fusarium moniliforme* agree with Sheldon's description (6) of this organism.

The size and shape of the perithecia, asci, and ascospores, absence of paraphyses, and the predominance of the 1-septate ascospores of the fungus also agree with Wineland's (8) descriptions of these characters for *Gibberella moniliformis,* the perfect stage of *Fusarium moniliforme.*

Macroscopically, the perithecia of *Gibberella moniliformis* are very similar to those of *G. saubinetii* (Mont.) Sacc., another species, common on corn and wheat in the North. Perithecia of both species are scattered or gregarious and have a bluish appearance. Microscopically, the 2 species are separable on the basis of certain morphological characters. According to Wineland (8), the straight, usually 1-septate ascospores of *G. moniliformis,* constricted at the septa and rounded at the ends and measuring 15–19 × 3.9–4.8 μ, contrast sharply with the curved, 3-septate ascospores of *G. saubinetii,* scarcely constricted at the septa and pointed at the ends and measuring 18–24 × 4–5 μ, as given by Saccardo (5). The macroconidia of the 2 species are similar in some respects and at times are difficult to differentiate. According to Sheldon (6), the macroconidia of *G. moniliformis* are falcate, acute, usually 3-septate, and 25–40 μ long. Those of *G. saubinetii* are fusiform, curved, acute, apiculate, 5-septate, measuring 24–40 × 5 μ, as given by Saccardo (5). The microconidia of *G. moniliformis* are abundant, while those of *G. saubinetii* are very rare.

Production of the Perithecial Stage

Perithecia of *Gibberella moniliformis* have been found in Florida, occurring naturally on corn leaf sheaths. Wineland (8) produced perithecia of this fungus in pure culture by planting paired microconidial cultures of *Fusarium moniliforme* on media and also by combining 2 single-ascospore cultures.

In the present investigations 6 multisporous cultures originating from microconidia and macroconidia of *Fusarium moniliforme* were grown singly and in paired combinations with each other on potato-dextrose agar in Petri dishes. At the end of 65 days perithecia of *Gibberella moniliformis* were observed in 4 dishes in which paired cultures had been planted and were present only in that area on the agar where the cultures had grown in contact with each other. No perithecia were formed in dishes devoted to single cultures.

In July, 1931, cornstalks were inoculated with 9 multisporous conidial cultures of *Fusarium moniliforme*, singly and in paired combinations. This was done by making an incision about 2 in. long in the 4th or 5th internode of a stalk with a downward stroke of a scalpel. Two corn kernels, each having the same or a different culture of the fungus growing on it, were then inserted into the incision and then wrapped with a strip of nurseryman's tape. At the end of 30 days a single culture and 7 of the combination cultures had formed perithecia of *Gibberella moniliformis* on the inoculated stalks (Fig. 3, D).

In September, 1931, 18 corn leaf sheaths bearing numerous perithecia of *Gibberella moniliformis* were collected and a single ascospore from a single perithecium from each sheath was planted in a Petri dish or a test-tube containing potato-dextrose agar. At the end of 20 days several of these single-ascospore cultures produced perithecia, and within 30 days 15 of the 18 cultures had produced perithecia (Fig. 3). Single ascospores from perithecia produced in these cultures were planted and grown on agar and each produced perithecia abundantly, as well as microconidia and macroconidia.

Thus, it is demonstrated that the mating of the proper conidial cultures results in the production of perithecia, that a single conidial culture may form perithecia, and that the majority of single-ascospore cultures produce fruiting bodies of the perfect stage.

PHYSIOLOGY

Growth on Media. The fungus grew well at 30° C. on potato-dextrose, oatmeal, rice, cornmeal, and Lima-bean agars. The microconidia and macroconidia varied greatly in size on the same media, as well as on different media. Microconidia were produced abundantly on all of the different

media, while macroconidia were abundant only on potato-dextrose and oatmeal agars

Relation of Temperature to Spore Germination. Microconidia were germinated in water in drop cultures exposed to temperatures ranging from 5 to 39° C. in electrically controlled incubators. As noted in table 1, the optimum temperature for germination at the end of 24 hours appears to be at or near 30° C. Only 5 per cent of the microconidia exposed for 7 days at 11° C. germinated, while no germination occurred at 8° C. during the same period, thus showing that the minimum temperature for germination lies between 8 and 11° C. The maximum was not ascertained but would seem to lie between 35 and 39° C.

TABLE 1.—*Percentage of germination of microconidia of Gibberella moniliformis exposed to different temperatures for 24 hours*

Temperatures in degrees Centigrade	6	8	11	13	18	25	30	35	39
Percentage of germination	0	0	0	0	8	25	42	15	0

Relation of Temperature to Mycelial Growth. A series of 90 plates of potato-dextrose agar were inoculated in the center with uniform bits of fungous mycelium, and 10 plates were incubated for 5 days at each of 9 different temperatures ranging from 10 to 39° C. The diameters of the colonies were measured at the end of 1, 3, and 5 days, and the average diameter of the 10 plates at each temperature is presented in table 2. The optimum temperature for growth in this test was close to 30° C., the minimum between 10 and 14° C., and the maximum between 35 and 39° C.

Relation of Hydrogen-ion Concentration to Mycelial Growth. Gibberella moniliformis was grown on a 2 per cent potato-dextrose agar adjusted

TABLE 2.—*Relation of temperature to rate of mycelial growth of Gibberella moniliformis*

Degrees C.	Average diameter of colonies in centimeters		
	One day	Three days	Five days
10	0	0	0
14	0	1.7	2.5
19	1.0	3.1	4.5
24	1.3	4.2	6.4
28	1.6	5.3	7.2
30	1.7	5.6	8.4
33	1.4	3.5	5.3
35	0	1.9	3.0
39	0	0	0

to 9 different hydrogen-ion concentrations. Five test-tubes, each containing 15 cc. of this medium, were used for each concentration, and the concentrations used ranged from pH 2.1 to 8.1. The adjustments were made by adding the required number of drops of 2N and N/2HCl and N/2 NaOH, the determinations being made with a Youden Hydrogen-ion Concentration Apparatus. After the medium was adjusted it was poured into Petri dishes, inoculated in the center with uniform bits of mycelium, and exposed to a constant temperature of 30° C. for 6 days. According to the increase in diameters of the colonies, as given in table 3, the reaction most favorable for growth was pH 8.1, which probably is very near the optimum. Growth was slow at pH 2.6 and was inhibited at pH 2.1.

TABLE 3.—*The relation of hydrogen-ion concentration of potato-dextrose agar to the rate of mycelial growth of Gibberella moniliformis at 30° C.*

pH	Average diameter of colonies in centimeters		
	Two days	Four days	Six days
2.1	0	0	0
2.6	0	0	1.5
3.6	1.3	2.3	3.5
4.2	1.8	3.2	5.5
4.8	2.3	4.3	6.8
6.4	2.5	5.3	7.8
6.7	2.9	5.8	8.6
7.1	3.1	6.1	8.9
8.1	3.2	6.3	9.0

Pathogenicity

Ears of corn in Florida are seldom found free from infection with *Gibberella moniliformis*. It usually occurs in or on the kernels, and, when other organisms are present, *G. moniliformis* is usually present also.

In January and February, 1931, a test was made to determine the effect of the presence of *Gibberella moniliformis* on the viability of kernels of seed ears of 4 varieties selected from the 1930 crop. This test was made by removing 10 kernels from each ear and growing them in a modified rag-doll germinator. The results are given in table 4. The ears classified as having all strong kernels were those whose kernels formed radicles and plumules of normal size. Ears classified as having weak kernels were those whose kernels developed small pumules without radicles, small radicles without plumules, or plumules and radicles much smaller than strongly-germinating kernels. Ears with dead kernels were those whose kernels did not form radicles and plumules. *Gibberella moniliformis* was present on all dead

TABLE 4.—*The effect of Gibberella moniliformis on the viability of corn kernels germinated in a modified rag doll*

Variety	No. ears tested	Percentage of ears showing			
		All strong kernels	One or more weak kernels	One or more dead kernels	One or more weak and dead kernels
Whatley	229	35	33	20	12
Tisdale	73	56	16	16	12
Cuban Yellow Flint	64	54.5	20.6	11.6	13.3
Powell's White Dent	243	60.8	26	8	5.2

kernels, all those that were weak, and on most of those that showed a normal germination. Since the only organism present was *G. moniliformis*, it is reasonable to assume that it was responsible for the death and reduced vigor of the seed. Its presence on the kernels that germinated indicates strongly that there are nonpathogenic strains of the fungus.

Kernels from ears that germinated 100 per cent in the modified rag-doll germinator were surface sterilized in a 1: 1000 bichloride of mercury solution and planted in sterilized soil inoculated with pure cultures of *Gibberella moniliformis* growing on sterilized corn meal. At the end of 8 days after the seedlings emerged from the inoculated soil their tops were yellowed and wilted, and the mesocotyls and roots invaded by the fungus were brown and shrunken. In most cases infection resulted in the death of the plants within 2 weeks after their emergence from the soil, and seedlings that were

TABLE 5.—*Yields of corn from good seed and seed weakened by infection with Gibberella moniliformis in Florida in 1929 and 1930*

Year	Location of experiment	Number of replications	Yield per acre		Difference per acre due to good seed
			Good seed	Seed weakened by *G. moniliformis*	
				Bu.	*Bu.*
1929	Gainesville	10	21.2	16.1	+5.1
	Quincy	8	48.3	44.8	+3.5
	Campbellton	10	17.8	17.2	+0.6
1930	Gainesville	8	11.9	12.1	−0.2
	Quincy	10	54.9	49.4	+5.5

not killed were much retarded in growth. Plants grown in noninoculated soil had healthy roots and a normal top growth.

Experiments were conducted in 1929 and 1930 to determine the effect on the yield of plants grown from seed weakened by infection with *Gibberella moniliformis*. Such seed, when tested in the germinator, showed 1 or more weak kernels but never more than 1 dead one out of each 10 from each ear used. The good seed germinated 100 per cent strong. The yields in 1929 were obtained from 1-row alternating plots 50 ft. in length, replicated. Yields in 1930 were obtained from the center row of alternating 3-row plots, also replicated. The results, as reported in table 5, show that seed weakened by infection from *G. moniliformis* yielded less on the whole than did good seed. The reduction in yield ranged from −0.2 to 5.5 bu. per acre.

SUMMARY

Gibberella moniliformis is one of the most prevalent corn-seed parasites in Florida. The outstanding symptoms of the disease caused by it are discussed.

The morphological characters of the imperfect and perfect stages of the species of Gibberella investigated agree with the original descriptions given for *Fusarium moniliforme* Sheld. and *G. moniliformis* (Sheld.) Wineland.

The perfect stage was found occurring naturally on corn and was produced on media in pure culture by pairing multisporous cultures derived from microconidia and macroconidia and also by inoculating cornstalks with multisporous cultures singly and in combination. Single-ascospore cultures isolated from corn-leaf sheaths produced perithecia abundantly.

The fungus grew well on standard culture media but did best on potato-dextrose agar, in which it produced both microconidia and macroconidia abundantly.

Microconidia of *G. moniliformis* germinated in water at temperatures ranging between 11 and 35° C., the optimum being close to 30° C. In pure culture on potato-dextrose agar the minimum temperature for mycelial growth of the organism is between 10 and 14° C., the maximum lying between 35 and 39° C. and the optimum near 30° C.

Growth did not occur on potato-dextrose agar at pH 2.1, and the fungus grew best at pH 8.1. The optimum, however, may lie above this, as it was not grown on media at higher concentrations.

Modified rag-doll germination tests of seed ears showed that *G. moniliformis* either weakened or killed the kernels or had no effect on them. The roots and mesocotyls of corn seedlings grown in sterilized soil inoculated with *G. moniliformis* were invaded by this organism and the plants either died or were retarded in growth. In plot tests conducted in 1929 and 1930

the yields from seed infected with this organism were reduced from −0.2 to +5.5 bu. per acre.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

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THE PRODUCTION OF A WHITE FERTILE SALTANT OF HELMINTHOSPORIUM SATIVUM BY MEANS OF ULTRA-VIOLET RADIATION[1]

F. J. GREANEY AND J. E. MACHACEK

(Accepted for publication July 18, 1932)

Most of the previous investigations on the effects of ultra-violet radiation on fungi have been concerned with the effect on sporulation and with the determination of the fungicidal action of these rays. As far as the writers are aware, there is no published account of saltation having been induced by this means.[2] The purpose of the present paper is to give the results of studies undertaken to determine the effect of ultra-violet light on the rate of growth and on the frequency of saltation in *Helminthosporium sativum* Pammel, King, and Bakke.

The strains of *Helminthosporium sativum* investigated were isolated from diseased parts of cereal plants in 1930 and 1931. Single-spore isolations were made from stock cultures. From these monosporous cultures subcultures were made on plates of Bacto potato-dextrose agar (20 cc. per plate). The subcultures were placed in a dark incubator maintained at from 22°–24° C. and allowed to grow for 3 days before irradiation was begun.

The source of the ultra-violet light used in these studies was a quartz mercury-arc lamp (Alpine Sun Lamp, A.C. equipment, 110 volts, 60 cycle). The exposures were made at a distance of 35 cm. from the arc. The cultures were irradiated for a 4-minute period on 3 successive days. In 2 experiments a number of 3-day-old cultures were irradiated through ordinary Jena Petri-dish lids, while others were irradiated through vitaglass covers. The vitaglass covers were sterilized before substituting them for the regular Petri-dish lids. Control cultures were held for an equal length of time in the diffused light of the laboratory and at the same temperature as the irradiated cultures. Several monosporous strains of *Helminthosporium sativum* were used in each experiment.

It was found that comparatively short exposures to ultra-violet rays markedly affected some strains of *Helminthosporium sativum* with respect to the rate of growth and the frequency of saltation, while other strains were apparently unaffected. Nonirradiated cultures remained constant

[1] Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

[2] Since this paper was written, Dr. Hugh Dickson has published an account of "The effects of X-rays, ultra-violet light, and heat in producing saltants in *Chaetomium cochliodes* and other fungi." Ann. Bot. 46: 339–404. 1932.

and maintained their cultural characteristics. The greatest number of saltants was produced in cultures irradiated through vitaglass, while the number was less in those cultures treated through ordinary Jena Petri-dish lids. Similarly, ultra-violet rays had a greater or less effect on growth rate, depending on whether irradiations were made through vitaglass or Jena Petri-dish covers. It is apparent, therefore, that the most effective rays are limited to definite regions of the spectrum. The activating region of the spectrum has not been determined. In general, the effects of ultra-violet light on susceptible strains of *H. sativum* were a marked suppression of growth, an increase in pigment, and an increase in the rate of saltation.

Most of the saltants of *Helminthosporium sativum* induced by ultra-violet radiation were tested to determine, to some degree at least, their constancy. The saltants have remained true to type. Many seemingly sterile strains, as well as actively sporulating strains, were obtained. In every case, the formation of sterile strains was closely correlated with the loss of color. This character correlation was observed by Stevens[3] in *H. sativum*. Studies to determine the degree of stability of these new strains of *H. sativum* are being continued.

One strain of *Helminthosporium sativum*, designated as R. L. No. 9, was very unstable in culture and saltated freely when exposed to ultra-violet rays. The original culture was obtained from a rotted crown of Marquis wheat. A single conidium isolated from it in February, 1932, gave rise to a culture from which a number of subcultures were made on plates of Bacto potato-dextrose agar and irradiated in the usual manner.

Four days after the final irradiation had been made 5 saltants, which differed very markedly from the parental colony, appeared as sectors in 3 subcultures irradiated through vitaglass. Transfers from these sectors and from the parent colony were made to freshly-poured plates. Mycelium from one of these saltants gave rise to a culture producing only hyaline mycelium and almost colorless conidia; whereas transfers from the parent form gave typical dark mycelium and dark-brown conidia (Fig. 1). Slight differences in morphology and physiology, as well as in growth rate, of the saltant and parent strain were also observed. This albinistic form has been subcultured repeatedly (both from mycelium and conidia) and has been irradiated but has remained unchanged. There seems to be no doubt, therefore, that an albinistic strain of *Helminthosporium sativum* has been permanently established by ultra-violet radiation.

Although there was a distinct loss of color in the saltant, no real difference in pathogenicity between the saltant and its parent was noticeable.

[3] Stevens, F. L. The *Helminthosporium* foot-rot of wheat, with observations on the morphology of *Helminthosporium* and on the occurrence of saltation in the genus. Ill. Nat. Hist. Surv. Bul. 14: 77–185. 1922.

This may be due to the very inadequate means so far available for measuring slight differences in pathogenicity in this organism. From figure 2 it is apparent that the parasitic behavior of the saltant C does not markedly differ from that of the parent form A, from which it was derived. It should be stated that their relative pathogenicity has been tested only to the leaves of young barley and wheat plants grown in the greenhouse. Thus far, no further comparison in pathogenicity has been made between the normal form and the saltant. In order to verify the parasitic behavior of the parent and its white saltant, reisolations were made. In each case the respective organism was obtained.

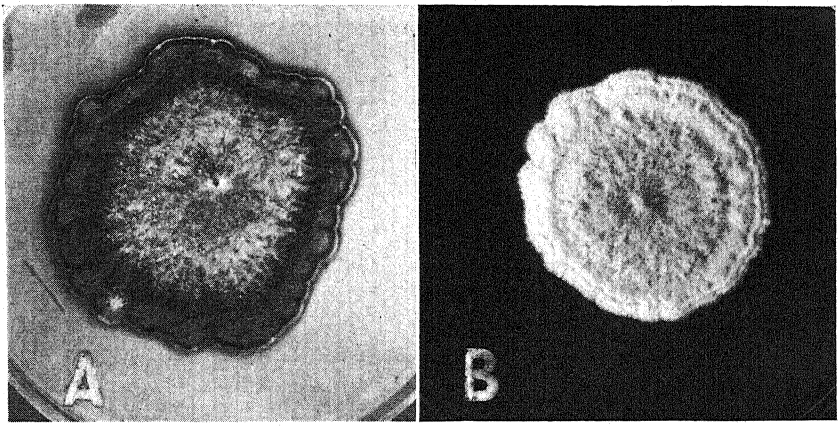


FIG. 1. *Helminthosporium sativum.* Surface views of 10-day-old colonies. A. Dark parent. B. White saltant produced by ultra-violet radiation.

It appears from the studies of many workers that the inherent parasitic capabilities of strains of pathogenic fungi do not change readily. Apparently, most mutations in fungi have been due to the loss of a factor or a group of factors for color. It is of interest that the first conclusive demonstration of mutation in *Puccinia graminis tritici* Eriks. and Henn. was a color mutation. This was reported by Newton and Johnson[4] in 1927. Since that time, however, Stakman, Levine, and Cotter[5] have reported the occurrence in *P. graminis tritici* of a mutation for pathogenicity. It has, therefore, been assumed by the writers that new forms of *Helminthosporium sativum,* differing pathogenically from any forms hitherto known, may occur. The importance of light as a factor in the induction of fungal saltants in nature is relatively unknown, but it appears important enough,

[4] Newton, Margaret, and Thorvaldur Johnson. Color mutations in *Puccinia graminis tritici* (Pers.) Eriks. and Henn. Phytopath. **17**: 711–725. 1927.

[5] Stakman, E. C., M. N. Levine, and R. U. Cotter. Origin of physiologic forms of *Puccinia graminis* through hybridization and mutation. Sci. Agr. **10**: 707–720. 1930.

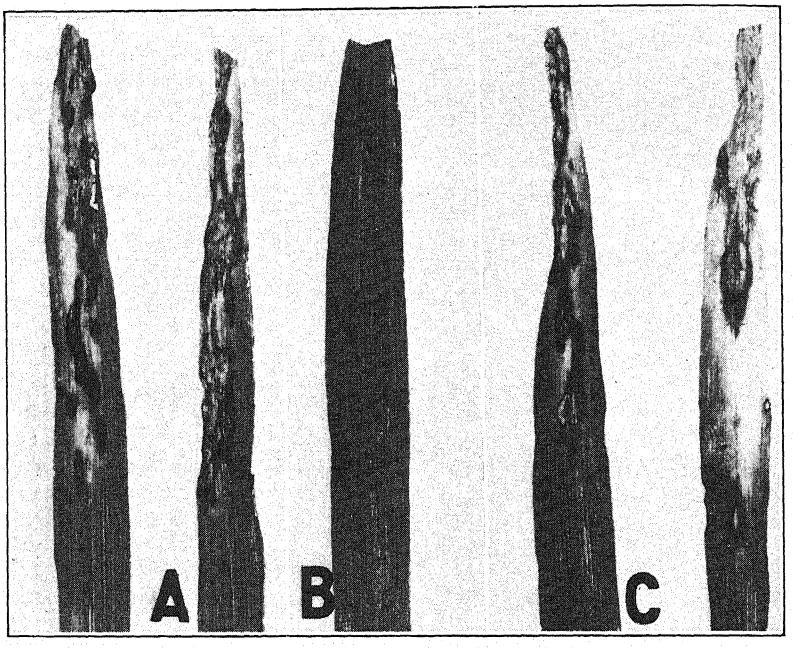


FIG. 2. Leaves of Trebi barley, showing comparative virulence of strains of *Helminthosporium sativum*. A. Dark parent. B. Noninoculated control. C. White saltant.

as shown by the results presented here, to merit further and more detailed investigation.

From a taxonomic view point, the discovery of a strain of *Helminthosporium sativum* with white mycelium and almost colorless conidia is of interest, especially in view of the fact that it is an example of a saltation so great as to remove this form completely from the Helminthosporium group, as described in the taxonomic classification of the *Fungi Imperfecti*. The classification of this new white fungus presents unusual difficulties.

The description of the form-genus Helminthosporium distinctly specifies that the spores or the mycelium are dark. The newly-discovered strain has hyaline mycelium and almost colorless spores, but the spore mass and the mycelium together produce a pale salmon color. As the parentage of this fungus is definitely known, it is convenient to accept it as an albinistic form of *H. sativum*. If this new strain were isolated from diseased plants in nature, it would not in all probability be recognized as a Helminthosporium.

Although white sterile strains of *Helminthosporium sativum* have been frequently obtained in artificial culture by many workers, as far as is known to the writers these strains have never been obtained through direct isola-

tion from diseased plants. If a sterile strain were isolated from a living host, its identity would be difficult to establish by microscopical examination, and, beyond doubt, physiological tests, such as "serum diagnosis," would be required to establish its relationships. It is questionable if even such special tests would be sufficient to identify the fungus.

SUMMARY

A preliminary study has been made of the effect of ultra-violet light on the frequency of saltation in *Helminthosporium sativum*. Cultures of several monosporous strains of this fungus were exposed to the action of ultra-violet light for a 4-minute period on 3 successive days. A few of the strains were apparently unaffected by the rays; the majority produced saltants. The saltants so far have remained constant. In addition to a decided increase in the frequency of saltation, the radiation produced a marked suppression of growth and an increase in pigmentation in those strains affected by the rays.

One strain of *H. sativum* irradiated with ultra-violet light produced a saltant that differed from the parent strain in that the mycelium is hyaline and the conidia almost colorless. In pathogenicity it does not differ appreciably from the parent strain. The difficulty of fixing the taxonomic position of this strain, were it found in nature, is noted.

DOMINION RUST RESEARCH LABORATORY,
WINNIPEG, MANITOBA, CANADA.

OCCURRENCE AND PATHOGENICITY OF NEMATOSPORA SPP. IN FLORIDA

GEORGE F. WEBER

(Accepted for publication June 11, 1932)

Diseased sweet-pepper pods, collected in the vicinity of Miami, Florida, May 20, 1926, contained an organism that, in pure culture, proved to be a species of Nematospora, identical with the illustrations and descriptions of *Nematospora coryli* Peglion described by Peglion (14), as occurring on hazelnuts in Italy. Further work on the organism was not possible at the time and the culture was lost.

On January 10, 1932, H. S. Fawcett found a diseased Satsuma orange on the Florida Agricultural Experiment Station grounds and demonstrated the presence of a species of Nematospora in the affected tissue. He considered it to be identical with the organism previously found by Lee (10) and Fawcett (5) in citrus and other hosts in the Orient and in California. Further collections were made by the writer in a small planting of young bearing satsuma trees, and, by actual counts, 1½ per cent of the fruit on these trees showed such external symptoms as to make it possible to detect the disease. On November 19, 1932, *Nematospora coryli* was found infecting oranges and grapefruit in Pinellas County and, on November 24, on tangerines in Alachua County. The purpose of this paper is to report the occurrence of certain *Nematospora* spp. in the United States and Florida and the results of inoculation experiments.

The infected satsuma fruits always showed more or less drying out in local areas of the rind. The tissue between the oil glands in the affected areas was sunken, causing the oil glands to protrude slightly above the surface. Usually the rind was somewhat wrinkled in the areas invaded by the organism. Not all such fruits, showing the above symptoms, however, were infected. Upon removing the rind, one or more locules showed the infection. In recently infected fruits there were plainly visible whitish spots, circular or irregular in shape and of various sizes located in the locule covering. In older infections the juice sacs within the locule were affected. They showed loss of moisture and often a collapsed condition. In the latter instances one end of the juice sac adhered to the locule wall. Often, in old infections, a discoloration was evident on the locule covering and in the ends of the juice sacs in contact with it. At first this coloration is a lighter color than the noninvaded tissue but later it becomes darker and, when most of the natural moisture has disappeared, the tissue is a bright reddish brown, as observed on other citrus by Fawcett (6). *Nematospora coryli* has been isolated from all stages just described.

While these investigations were in progress a large number of isolations were made from naturally infected satsumas. In a single instance, on February 8, 1932, *Nematospora gossypii* Ashby and Nowell was obtained from a satsuma that had all the characteristics of the disease caused by *N. coryli*. This organism infected the fruit to the exclusion of *N. coryli*. This is, so far as known, the first time *N. gossypii* has been reported outside of the geographical limits of the Lesser Antilles and Trinidad (2), Nigeria (9), and Burma (15). This is also the first report of this organism on citrus fruit of any kind, so far as the writer can determine, although it has been reported previously on cotton, *Datura* sp., and milkweed. Although the diseases caused by *N. gossypii* and *N. coryli* are indistinguishable, the organisms, in culture, are very distinct, as pointed out by Ashby and Nowell (2), Guilliermond (8), and Fawcett and Lee (7).

A single ripe tomato fruit was collected on February 7, 1932, near Homestead, Florida, infected with *Nematospora coryli*. A second shipment of tomatoes received from that section of Florida on February 17 showed 3.6 per cent infected fruit. Another shipment, about March 1, showed 1.3 per cent. The infected fruits in the second and third shipments were in different stages of ripeness, namely, green, pink, and red, indicating that infection took place while they were green. This fungus has been previously reported from tomatoes grown in southern California, Cuba, and Mexico, by Schneider (16, 17), but was named *Nematospora lycopersici*, which is now considered a synonym of *N. coryli* (2). Excepting Schneider's report of its occurrence in southern California, this is the only report of this disease on tomatoes from the United States, although it has been reported on lima beans in Virginia by Wingard (19, 20, 21) and in Illinois by Anderson (1) as *Nematospora phaseoli*, a synonym.

The infections on tomatoes were always associated with a disease previously described (18) as "cloudy spot," which is now considered to be caused by sucking insects of the pumpkin and leaf-footed plant-bug group. Other investigators, Davy (4) and Nowell (12, 13) have intimated and Fawcett (6) has shown that sucking bugs undoubtedly have been the carriers of the *Nematospora* spp. However, in no instance have they isolated the organism from the mouth parts of certain of these insects, even when captured in the act of feeding. The writer made dilution plates of the mouth parts of a dozen individual bugs and obtained *Penicillium* spp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., and other fungi, bacteria, and yeasts, but no *Nematospora* sp.

The cloudy spots appear as lighter colored areas anywhere on the surface of tomato fruits. Examination of the tissue shows that it is somewhat pithy or as though the moisture had been withdrawn and the spaces filled with air. When these spots are infected (Fig. 1), the host tissue involved

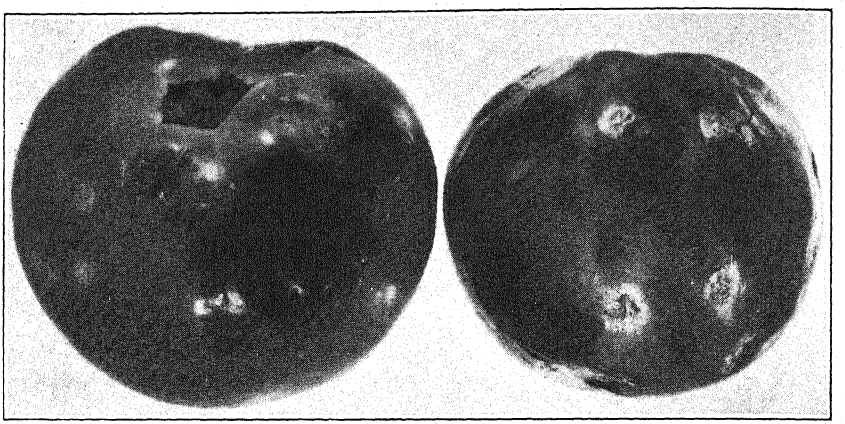


FIG. 1. Tomatoes naturally infected with *Nematospora coryli* and showing diseased areas.

does not ripen so rapidly as the noninvaded tissue, leaving green islands in a pink or red fruit. These green spots gradually darken as the infection spreads, become sunken, and slowly soften. They are finally invaded by secondary soft-rotting organisms.

Both *Nematospora coryli* and *N. gossypii* grow well on potato-dextrose agar and have been increased on that medium for use in inoculation experiments. Water suspensions were made from cultures of the organisms and were used for inoculating satsuma, tangerine, and tomato fruits in the laboratory. The cultures of *N. coryli* were isolated from satsumas and tomatoes and *N. gossypii* from satsumas. The suspensions contained vegetative cells, mycelium, and ascospores of each of the organisms.

The fruits to be inoculated were disinfected with corrosive sublimate (1: 1000) for 20 minutes; they were then thoroughly washed and placed in moist chambers. Before the inoculations were made the surface of the fruits was swabbed with 95 per cent alcohol and allowed to dry. A large loop transfer needle was then used to place several drops of the inoculum on the sterilized surface, after which a puncture was made with a small sterile needle through the drop of inoculum. The inoculum was usually sucked into the citrus fruits when the needle was removed, while the tomato fruits often exuded juices in droplet form that mingled with the inoculum until the moisture evaporated. Each fruit was inoculated in 10 places and placed in a moist chamber immediately. After 24 hours the tops of the moist chambers were removed and the fruits were left in the open dishes on the laboratory shelf. The checks were similarly treated, except that sterile distilled water was used in place of the inoculum. After 2 weeks the fruits were examined and the data obtained are shown in table 1.

TABLE 1.—*Results following inoculations of satsumas, tangerines, and tomatoes with Nematospora coryli and N. gossypii*

Kind and source of inoculum	Fruits	Inoculated	Diseased
Nematospora coryli (satsuma)	tomato	20	20
	satsuma	12	12
	tangerine	12	10
Nematospora coryli (tomato)	tomato	20	20
	satsuma	12	12
	tangerine	12	12
Nematospora gossypii (satsuma)	tomato	20	16
	satsuma	12	9
	tangerine	12	7
Sterile distilled water	tomato	3	0
	satsuma	3	0
	tangerine	3	0

The area immediately around the points of inoculation dried rapidly and became discolored and shrunken. The culture of *Nematospora coryli* from tomatoes appeared to cause infection of satsumas and tangerines more rapidly than the strain isolated from satsumas. On tomatoes, however, there was very little difference in the effect of the two strains. Both of them produced colonies within the seed cavities and caused a discoloration of the seeds. The multiplication of the organisms was much more rapid in tomatoes than in satsumas or tangerines.

The development of *Nematospora gossypii* in tomatoes was somewhat different from that of *N. coryli* in that it lacked free floating vegetative cells and showed extensive mycelial growth. In this case the mycelium invaded more extensive areas of the inoculated tomato, even growing in among the hairs of the seeds. Ascospores of both species were produced in abundance in infected fruits. Successful inoculations with *N. gossypii* were not so certain as with *N. coryli*. This was also the experience of Marsh (11) in inoculating cotton bolls, although Dastur and Singh (3) obtained a high percentage of infection of cotton bolls with *N. nagpuri* Dast. The organism was recovered in each case of the successful inoculations.

SUMMARY

Nematospora coryli was first found in Florida infecting sweet-pepper pods. It was recently found in this State on satsuma oranges by H. S. Fawcett and on tomatoes, grapefruit and oranges by the writer.

Nematospora gossypii was found on the satsuma orange in Florida for the first time and is the first report of *N. gossypii* in the United States on any host.

This constitutes the first report of both of these organisms on the satsuma orange and of *N. coryli* on peppers in the United States.

Inoculations show both species to be pathogenic on satsuma, tangerine, and tomato.

The isolations of the organisms from tomato and one of those from the satsuma orange are indistinguishable and undoubtedly are *N. coryli*.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

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APPLICATION OF THE TERMS "ANTHRACNOSE" AND "SCAB" TO PLANT DISEASES CAUSED BY SPHACELOMA AND GLOEOSPORIUM

ANNA E. JENKINS[1]

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INTRODUCTION

This paper presents the history of the term anthracnose and makes certain suggestions for the use of this and the term scab, particularly as applied to diseases caused by Sphaceloma (including Elsinoë) and Gloeosporium (including Colletotrichum). The treatment is mainly from the standpoint of phytopathologists.

HISTORY OF THE TERM ANTHRACNOSE

Originated to Designate a Sphaceloma Disease of Grapes. To avoid confusion in the term *charbon* as then used in France to designate both a smut of cereals (Gramineae) and the disease of grapes (Vitis) now known to be caused by *Sphaceloma ampelinum* de Bary, Fabre and Dunal (12), in 1853, substituted for the latter disease a new name of the same meaning: "anthracnose (ἄνθραξ, *charbon, et* νόσος, *maladie*)." This is the generally accepted name for the grape disease, although, as listed by Weinmann (38), many other terms besides those named have been applied to it in France and elsewhere. The term anthracnose was thus first used in a symptomatic sense to signify only this one disease, which is hyperplastic in type. *Sphaceloma ampelinum,* described (3) in 1874, was until recently the only species in the form genus Sphaceloma. Burrill (7), Scribner (27), and others considered *Gloeosporium ampelophagum* Sacc. (24) to be the same organism, and this identity was recognized by Saccardo (25, p. 267) in 1906. The classification of the fungus in the genus Gloeosporium led to its being treated as of this genus in many European and American publications and herbaria.[2]

Application to Related Diseases. In accordance with Burrill's (7) discovery of the probable congeneric relationship of the pathogen of what was then known as "blackberry and raspberry cane rust" with *Sphaceloma*

[1] Much of the subject matter presented in this paper is part of a thesis presented to the Graduate School of Cornell University, in June, 1927, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Thanks are due H. M. Fitzpatrick, C. M. Massey, and A. J. Eames, under whose direction the work was done. Acknowledgment is also made to M. F. Barrus, H. S. Fawcett, H. R. Fulton, W. A. McCubbin, C. L. Shear, H. H. Whetzel, and R. P. White for a critical reading of this paper, especially the parts dealing with suggestions for the application of the terms anthracnose and scab to unnamed diseases.

[2] Observations with respect to European herbaria in part by H. S. Fawcett.

ampelinum, Scribner (29) suggested the name "anthracnose of raspberry and blackberry" for this bramble disease. He (29) explained that the new name was "proposed . . . in uniformity with anthracnose of the vine caused by another species of the same genus." Identifying (29) the bramble-disease pathogen as *Gloeosporium venetum* Speg., he continued to treat the fungus as of the genus Gloeosporium (of which the conidia are generally larger than those of the genus Sphaceloma), although he (27, 28) referred to the pathogen of grape anthracnose as of the genus Sphaceloma. His (30, Figs. 1511 and 1578) illustrations showing the "striking similarity" of these fungi are reproduced in figure 1. Until Burkholder's (4, 5)

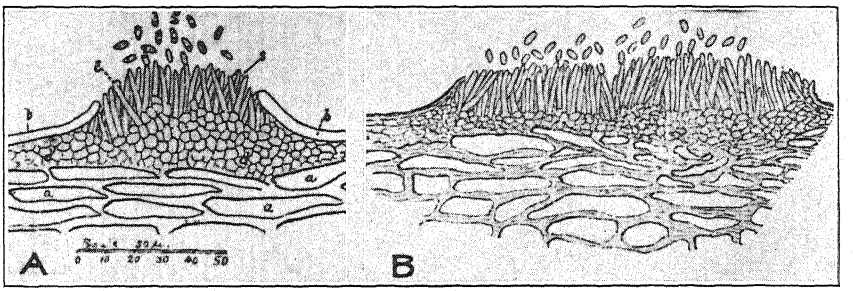


FIG. 1. Conidial fructifications of (A) *Sphaceloma ampelinum* and (B) *Gloeosporium venetum* as illustrated by Scribner (30). These show, as Scribner stated, "the striking similarity of the two fungi."

discovery of the perfect stage of this fungus in 1914, when it was classified as *Plectodiscella veneta* (Speg.) Burk., the bramble fungus was called *G. venetum* and its relationship to *S. ampelinum* rarely mentioned. The two organisms have recently been classified in the genus Elsinoë, the grape fungus as *E. ampelina* (de Bary) Shear (31), the bramble as *E. veneta* (Speg.) Jenkins (16).

The term anthracnose has been applied to four other diseases caused by Elsinoë or Sphaceloma, those affecting rose (Rosa) (17), apple (Malus) and pear (Pyrus), snowberry (*Symphoricarpos albus* Blake var. *laevigatus* (Fernald) Blake), and Labrador tea (Ledum). Cobb (8, opposite page 646) designated the rose disease anthracnose presumably because he thought at the time that its pathogen was a Gloeosporium. As affecting pear, the apple and pear disease was so named by Peglion (20) evidently because of the resemblance of the leaf symptoms to those of grape anthracnose. The early suggestion (36, pp. 393–394) that the snowberry-disease pathogen might be a Gloeosporium doubtless led to the disease being called an anthracnose when it was first reported (36). Barrus and Horsfall (2) later used the same name for the disease, with the explanation that this was

done because of the resemblance of the pathogen to *Elsinoë veneta,* then known as *Plectodiscella veneta.* Zeller and Deremiah (41) did not explain why they applied the term to the Labrador tea disease, but, judging from their paper, this was done because the pathogen is of the same genus (Elsinoë) as that of grape anthracnose.

Application of the Term Anthracnose to Diseases Caused by Gloeosporium and Colletotrichum. In the report containing Scribner's (29) account of anthracnose of raspberry and blackberry, he also discussed anthracnose of the bean (Phaseolus), identifying its pathogen as *Gloeosporium lindemuthianum* Sacc. and Magn. The organism was later transferred to the genus Colletotrichum, as *C. lindemuthianum* (Sacc. and Magn.) Br. and Cav.[3] on the basis of Scribner's (29) observation that it produced setae. This bean disease is often given as an example of a typical anthracnose as well as of a strictly necrotic disease. Scribner (29) apparently applied the term anthracnose to this disease in conformity with the term as used for bramble anthracnose, of which the pathogen was also classified in the genus Gloeosporium. His application of the term to these two diseases constitutes early, if not the first, use of the word for other than the grape disease for which it was originated.

With apparently little thought of its origin, the word has since been in general use for diseases caused by species of Gloeosporium and Colletotrichum and a few genera other than Sphaceloma and Elsinoë. It has also been used to refer to the pathogens of diseases that have been termed anthracnoses. Examples of the use of the term as discussed in this and the preceding paragraph are given below.

According to Stoneman (37), the term anthracnose was "first applied to the 'bird's-eye' fungus of the grape (*Sphaceloma ampelinum* De Bary . . ." and "has since been applied to diseases having similar external appearance and agreeing in general microbotanical characters." Edgerton (11) said that "some [here possibly referring to Shear and Wood (32)] would limit the use of the word anthracnose to those fungi having an ascigeral stage as in the genus Glomerella; but I prefer to use it as commonly used, for all fungi having a Gloeosporium-like conidial stage." He asserted (11, p. 394) that the "majority of the anthracnoses that have been described belong to the genus Glomerella." Shear and Wood (33) said that "in current usage the term 'anthracnose' is applied to diseases caused by fungi belonging to a few other genera besides Glomerella, but that it would be better if the name . . . were restricted to the diseases caused by Glomerella." This genus, they stated (33), is "usually referred to in its conidial stage as Gloeosporium and Colletotrichum."

[3] Briosi, G., and F. Cavara. I fungi delle piante coltivate ed utili, Fasc. 2, No. 50, 1889.

The use of the term anthracnose in naming diseases caused by Gloeosporium and Colletotrichum is further indicated by Rorer's (23) statement that "like other anthracnoses," anthracnose of the mango (Mangifera) "is caused by a fungus belonging to the genus Gloeosporium or Colletotrichum." It is shown also by the fact that the pathogens of most of the 48 anthracnose diseases referred to by Stevens and Hall (34) belong to these two genera and also by Wolf's (40) naming of the vetch (Vicia) disease caused by *Protocoronospora nigricans* (Atk. and Edg.) emend. Wolf "false anthracnose" to distinguish it from what he virtually called the "true anthracnoses" (diseases caused by Gloeosporium and Colletotrichum).

In the 1911 edition of the Century Dictionary[4] the term is defined as referring only to grape anthracnose, "A disease of grape-vines which affects the leaves, young stems, and green berries and is caused by the fungus, *Sphaceloma ampelinum.*" In the 1930 edition of the New Century Dictionary,[5] however, the definition is much more general: "A destructive disease of grape-vines, in which brown spots appear on the shoots, leaves, and berries, due to the fungus, *Sphaceloma ampelinum;* also, any of the various other plant-diseases due to fungi." The 1931 edition of Webster's Dictionary[6] defines the term as "A common plant disease caused by several species of Melanconiaceous fungi."

APPLICATION OF THE TERM SCAB TO THESE DISEASES

Although not in common use for them, the term scab, as well as that of anthracnose, has been applied to the diseases of grape (19) and brambles (6) caused by Elsinoë and to the bean disease caused by *Colletotrichum lindemuthianum* (1).

The term is commonly used for the disease of citrus (Citrus) and avocado (*Persea americana* Mill.) (13), caused by Sphaceloma, and those of Lima bean (*Phaseolus lunatus macrocarpus* Benth.) and sword bean (*Canavalia gladiata* (Jacq.) DC.) caused by species of Elsinoë. Scribner (26) named the citrus disease scab obviously because of its hyperplastic scab-like lesions. Stevens (35) called the avocado disease scab on account of its "many points in common" with citrus scab. Following the precedent of Scribner (26), the writer (14, 15) applied the term to the two legume diseases, that affecting Lima bean having already been called "verruga" by Cuban growers of the crop.

[4] Century Dictionary. An Encyclopedic Lexicon of the English Language, prepared under the superintendence of William Dwight Whitney . . . rev. and enl. under the superintendence of Benjamin E. Smith. 1911.

[5] New Century Dictionary of the English Language. Edited by H. G. Emery and K. G. Brewster . . . vol. 1, 1929.

[6] Webster's New International Dictionary of the English Language based on the International Dictionary of 1890 and 1900. Springfield, Mass., U. S. A., 1931.

SUGGESTIONS FOR THE FUTURE USE OF THE TWO TERMS

The general statement may be made that plant diseases are classified either on the basis of the taxonomic position of their pathogens or on their predominating disease processes. Whetzel (39), as well as others, maintains that the latter basis is the "only logical" one. According to him, the etiological basis is objectionable because there is no "consistent correlation between kinds of pathogenes involved and the character of the disease they produce."

As already explained, in naming plant diseases, the term anthracnose is now used chiefly in an etiological sense, although it was originally used only in a symptomatic sense. In the future, where this term and that of scab are applied by pathologists to as yet unnamed diseases of the groups discussed herein, it is suggested that they be used in a symptomatic sense, anthracnose for necrotic and hypoplastic diseases having somewhat restricted lesions as in bean anthracnose and scab for diseases having hyperplastic scab-like lesions as in citrus scab. The diseases of Antidesma and Tinospora (22, t. 1) caused by Elsinoë, for example, which are hyperplastic in type, could thus appropriately be called scab, and the Sphaceloma disease of poplar (Populus) (18), apparently non-hyperplastic, anthracnose.

Of course, neither term should be employed where already in common use for another disease of the same plant, or else the terms should be so qualified that confusion will not result. For other reasons, a different terminology may sometimes be desirable, as in cases where some other particularly descriptive name has been applied to a disease.

The recent application (9, 10) of the name anthracnose to a Gloeosporium disease of snowberry has resulted in a duplication of disease names of this suscept. The term "ripe rot" (21) serves to distinguish the Gloeosporium disease of grapes from that caused by Sphaceloma, and it is suggested that this name may appropriately be used for the snowberry disease caused by Gloeosporium, which also becomes evident upon the ripening of the fruits (10).

By common consent the name of a plant disease should not be used by itself to refer also to its pathogen or, as expressed by Whetzel (39), "disease and pathogene concepts" should not be synonymized. Therefore, it is desirable that the use of the term anthracnose in reference to pathogens of diseases so named be discontinued.

SUMMARY

The term anthracnose was originated by Fabre and Dunal for a grape disease, in order to avoid confusion in the term *charbon* as then employed in France to designate both a cereal smut and the grape disease. The new

name for the grape disease was used only in a symptomatic sense. The pathogen of this disease was later described as *Sphaceloma ampelinum* and is now classified in its perfect stage as *Elsinoë ampelina*. Scribner extended the term anthracnose to denote the related bramble disease the causative organism of which was commonly known in its imperfect stage as *Gloeosporium venetum* and recently classified in its perfect stage as *E. veneta*. He also applied the term to the unrelated legume disease caused by *Colletotrichum lindemuthianum*. As in these two instances, the term has since been applied chiefly in an etiologic sense to designate diseases caused by Elsinoë, Sphaceloma, Gloeosporium, and Colletotrichum and those caused by fungi of a few other genera. Some of these diseases, both hyperplastic and nonhyperplastic, have also been called scab.

It is suggested that, in the future, where this term and that of scab, may be used in naming diseases caused by fungi of the genera named, they be applied in a symptomatic sense, anthracnose for necrotic and hypoplastic diseases with somewhat restricted lesions, as in bean anthracnose, and scab for hyperplastic diseases with scab-like lesions, as in citrus scab.

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EXPERIMENTS WITH HEAD SMUT OF CORN IN WESTERN OREGON[1]

E. N. Bressman and H. P. Barss

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INTRODUCTION

Head smut of corn (*Sorosporium reilianum* (Kühn) McAlp.) has been observed in western Oregon for many years. It is of interest to note, however, that the disease, although widely scattered, does not occur in all places where corn is grown in this region but is localized in certain areas where the smut may be very severe in particular fields, while it is absent or unnoticed in the intervening areas even though soil and climate are very much alike and similar varieties of corn are grown.

In 1930 a serious outbreak ruined a field near Salem, attacking 90 per cent of the plants. This directed our attention to the possible serious economic consequences of the disease and led to the trials reported herein.

PREVIOUS RESEARCH

This head smut was first found in 1868 in Egypt and has since been noted in many parts of the world. It was first reported on the Pacific Coast from the State of Washington, in 1920, by Dana and Zundel[2] and from California in the same year by Mackie.[3] Among the most extensive studies of the disease are those reported by A. A. Potter[4] in 1914 and by Reed, Swabey, and Kolk[5] in 1917. The more important literature on the disease has been discussed in their contributions and will not be reviewed here.

The work of various investigators, particularly that of Reed and his associates, indicates that the head smut of corn does not readily attack sorghums, and *vice versa,* although the strains are morphologically indistinguishable.

Several workers have demonstrated that the disease may be transmitted to a certain extent by seed-borne spores, but Potter[4] and others have shown

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[2] Dana, B. F., and G. L. Zundel. Head smut of corn and sorghum. Washington Agr. Expt. Sta., Pop. Bul. 119. 1920.

[3] Mackie, W. W. Head smut in sorghum and maize. Phytopath. 10: 307–308. 1920.

[4] Potter, A. A. Head smut of sorghum and maize. Jour. Agr. Res. 2: 339–372. 1914.

[5] Reed, G. M., Marjorie Swabey, and Laura Alma Kolk. Experimental studies on head smut of corn and sorghum. Bul. Torrey Bot. Club 54: 295–310. 1927.

that the chief source of infection lies in smut-contaminated soil. Seedlings are attacked in the early stages of their development and the parasite becomes systemic, growing up within the plant and generally transforming some or all of the flowering parts into smut galls. Numerous instances are on record, however, where, instead of producing smut masses in the tassels and ears, the presence of the parasite induces the formation of abnormal vegetative proliferations in place of normal floral organs. It has also been noted by McAlpine[6] and by Reed, Swabey, and Kolk[7] that the tassels may sometimes show no smut on corn plants having infected ears, whereas if smut appears in the tassel, all the ears are generally smutted. These phenomena were observed in the trials reported herein.

Thus far, no satisfactory control measures for head smut seem to have been developed. Seed treatment of smut-contaminated grain has been reported effective in eliminating this source of infection, but no practical method has yet been worked out for the elimination of soil-borne infection, a vastly more important source of the disease. Potter,[8] however, suggests what he characterizes as "a possible, though very doubtful and as yet untried specific measure for prevention, *i.e.,* a treatment of the soil about the seed at planting time in some such way as is done for onion smut."

PRESENT INVESTIGATIONS

In view of the seriousness of the head smut of corn on various Oregon farms, it was thought desirable to carry out certain infection studies by means of greenhouse and field trials, using methods similar to those of previous workers, and, in addition, to try out various materials for seed treatment as well as for treatment of the soil at planting time, as suggested by Potter. The results here presented confirm and extend the knowledge contributed by others, but they are based on experiments so limited in scope that they cannot be considered conclusive in all particulars.

GREENHOUSE TRIALS IN 1931

Seed Treatment Experiments With Corn. Seed of the variety Minnesota 13, from the 1930 crop on the Oregon State College Farm, where head smut is unknown, was thoroughly coated with head-smut spores obtained from the badly infested field near Salem, referred to in the introduction. The contaminated seed was then divided into 8 lots, 6 of which were treated with various dust materials by shaking the seed vigorously with a liberal excess of the dust. With another lot, formaldehyde was used at the strength of 1 oz. to 1 gal. in a 15-minute soak. The treated lots and the nonsmutted and smutted controls were then planted in noncontaminated

[6] McAlpine, D. The smut of maize and its treatment. Jour. Dept. Agr. Victoria 8: 290–298. 1910.

[7] See footnote 5.

[8] See footnote 4.

soil in a deep greenhouse bench on January 5. One lot of clean seed was similarly planted, after which the surface of the soil was given a liberal coating of smut spores. The soil surface was kept moist by daily watering. Greenhouse temperatures ranging from 70 to 90° F. during the day prevailed throughout germination and growth. By extending the daily exposure to light through the use of electric illumination, these plants were forced to produce tassels and ears by May 10. The results of these trials are reported in table 1.

TABLE 1.—*Results of various treatments of seed of Minnesota No. 13 corn, artificially contaminated with spores of Sorosporium reilianum. Greenhouse trials, Corvallis, 1931*

Treatment	Normal stalks	Stalks with tassel and ears smutted	Stalks with only ears smutted	Stalks with abnormal ears only	Total stalks
Clean seed—check	8	0	0	0	8
Smutted seed—check	2	2	14	12	30
Copper carbonate (50%)	4	0	0	0	4
Formaldehyde (1 oz. to 1 gal.)	3	0	1	0	4
Improved Semesan Jr.	0	0	3	1	4
Landplaster (powdered gypsum)	4	0	0	0	4
Corona Merko (mercuric)	0	0	2	2	4
Field's Corn Dust (copper)	1	0	1	2	4
Paraformaldehyde (powdered)	4	0	0	0	4
Clean seed, smut on top soil	4	0	0	0	4
Totals	30	2	21	17	70

These tests clearly demonstrate that corn may become infected from seed-borne spores, since artificial smutting of the seed resulted in evident infection in 28 out of 30 plants. Clean seed produced only clean plants, even where a layer of smut was placed on the soil surface. All of the seed treatments appeared to reduce or eliminate the infection, with the exception of Improved Semesan Jr. and Corona Merko, neither of which, of course, has been recommended for smut prevention, although commonly used on corn. These tests are too limited to warrant any decision as to the relative merits of the different materials employed.

It is interesting that out of 40 infected corn plants only 2 showed smutted tassels, while 17 disclosed the presence of the parasite only by the production of abnormal vegetative proliferation in the ears without the formation of smut masses. (Fig. 1.)

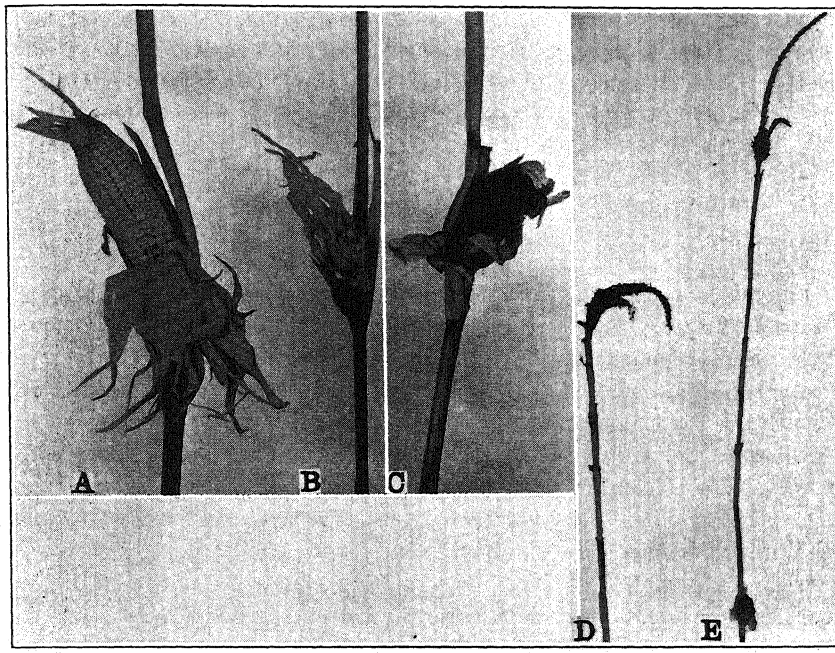


FIG. 1. Effect of *Sorosporium reilianum* on corn in greenhouse trials. A. Normal ear. B. Abnormal ear. C. Smutted ear. D. Stalk with smutted tassel. E. Stalk with smutted tassel and ear. In D and E leaves have been removed.

No conjecture is offered as to why the plants came through healthy in the lot where smut was placed on the soil surface at planting time, especially since considerable care was taken to keep the soil surface in a moist condition and the atmosphere of the greenhouse humid.

Infection Trials With Head Smut of Corn on Sorghum. Seed of 6 varieties of sorghum was obtained from G. M. Reed, of the Brooklyn Botanic Garden, and coated with head-smut spores from the same source as the material used for the above experiment with corn. The contaminated seed was planted in the greenhouse on February 17. The sorghum headed by July 28.

Dr. Reed reports on the relative susceptibility of these varieties to the sorghum strain of *Sorosporium reilianum* as follows: Black Amber sorgo (No. 48) susceptible; brown durra (No. 74) susceptible; Red Amber sorgo (No. 232) susceptible; Dawn Kafir (No. 186) resistant; Dwarf Yellow milo (No. 124) resistant; Manchu kaoliang (No. 191) resistant.

Although from 19 to 24 plants of each variety, 128 in all, matured, not a single plant showed any sign of head smut. This result confirms the prevalent opinion that the sorghums are not readily attacked by the ordi-

nary corn-infecting strains of *Sorosporium reilianum*. It would seem safe, therefore, to use sorghum in rotations on infested corn land where such procedure is practicable.

FIELD TRIALS IN 1931

The field experiments on susceptibility and soil treatment were conducted in the cornfields near Salem, Oregon, previously referred to, where most of the corn was ruined by the head smut in 1930. The soil is mapped as Holcomb silty clay loam. The corn was planted on May 8 in 26 single-row plots, 5 kernels to the hill.

Variety Trials. Six varieties of corn were planted without treatment to determine relative susceptibility in naturally contaminated soil. These were as follows: Minnesota No. 13 grown for nearly 25 years at the Oregon Agricultural Experiment Station and coming from a crop free of this disease; the "Schucking" strain, similar to Minnesota No. 13, obtained in the fall of 1930 from an infested field and probably carrying some seed-borne spores; Golden Glow obtained from a farm where no smut occurred in the crop; McKay Dent obtained from the college farm where no smut occurred; Putnam Red obtained from a farm where no smut was present; and African White, a flinty dent variety received from South Africa direct. Whether or not this came from a smut-free field is unknown. The results are shown in table 2.

TABLE 2.—*The results of attack by Sorosporium reilianum on 6 varieties of corn planted without treatment in smut-contaminated soil at Salem, Oregon, in 1931*

Variety	Stand	Total stalks	Disease-free stalks	Stalks with tassels and ears smutted	Stalks with only ears smutted	Stalks with abnormal ears only	Diseased stalks
	Per ct.						*Per ct.*
Minnesota No. 13	99	95	76	13	4	2	20
"Schucking"	95	92	57	20	13	2	38
Golden Glow	96	93	61	15	15	2	34
McKay Dent	83	80	65	4	8	3	19
Putnam Red	82	79	58	13	5	3	27
African White	78	75	50	17	2	6	33
Totals	—	514	367	82	47	18	28.6

The results shown in table 2 do not disclose any variety with marked resistance to head smut. The limited nature of the test and lack of replica-

tion do not allow any decision as to whether the different percentages of smut attack on the different varieties are true indications of relative susceptibility.

Field Treatment Trials. Two of the varieties, Minnesota No. 13 and the "Schucking" strain were given 10 treatments at the time of planting. Copper carbonate, containing 50 per cent copper; Corona Merko, a mercury-containing powder; Field's Corn Dust, a copper-containing powder; and Improved Semesan Jr., an organic-mercury dust, were applied in excess as dusts on the seed before planting. All of these dusts stick to the seed readily. Smuttox, composed of diatomaceous earth carrying 4 per cent formaldehyde; agricultural ground sulphur; and Superpoppy sulphur, which contains a considerable proportion of black gas by-product sulphur; were applied by placing about a level teaspoonful on the kernels after they were put in the hills. The hills were then covered. Formaldehyde was used at the strength of 1 oz. of the commercial (about 40 per cent) solution to 1 gal. of water. Germ-O-No liquid was diluted 1 to 10, and Garden Volck, a refined oil emulsion, was diluted 1 to 8. In these 3 liquid treatments the corn was planted and covered with the soil, after which the liquid was carefully poured on top of the soil. About 1 teacupful was applied to each hill, which was sufficient to wet the ground down to the kernels.

About 2 weeks after planting, stand counts were made and the hills that had more than 3 stalks were reduced to a uniform stand of 3. The growth for the first month was rather slow because of dry conditions. Timely rains in mid-June, however, were followed by rapid growth. The plants were left undisturbed until fall when counts of smutted, abnormal and normal tassels and ears were obtained. The results are reported in table 3.

The results presented in table 3 show that none of the treatments as carried out gave clear evidence of practical effectiveness for head-smut prevention in contaminated soil. Even the formaldehyde solution, so effective when applied to soil for onion-smut control, produced no definite evidence of protection as compared with the control rows. In the test with the "Schucking" strain, copper carbonate produced marked injury to stand, and additional trials have shown that, in excess quantity, copper carbonate inhibits the growth of the seminal roots. The formaldehyde-carrying dust, Smuttox, produced severe reduction in stand in both varieties. At the same time it afforded no protection. In passing it may be noted that the plots treated with Corona Merko, although showing considerable smut, appeared greener and more vigorous than any of the others from the seedling stage to harvest.

Taking the totals from all the field trials, which included 2,034 corn plants, 674, or 33 per cent, were diseased. Of these, 49 per cent showed both tassels and ears smutted, 40 per cent showed only ears smutted, while

TABLE 3.—*Field trials of the results of seed and soil treatments with corn against Sorosporium reilianum, conducted at Salem, Oregon, in 1931*

Treatment	Stand 2 weeks after planting	Total stalks matured	Disease-free stalks	Stalks with tassels and ears smutted	Stalks with smutted ears only	Stalks with abnormal ears only	Diseased stalks
			Minnesota No. 13				
	Per ct.						*Per ct.*
Copper carbonate	100	97	79	10	5	3	19
Corona Merko	89	86	53	16	14	3	37
Field's Corn Dust	77	74	48	13	12	1	35
Improved Semesan Jr.	100	99	61	18	15	5	38
Smuttox	30	29	20	4	4	1	31
Agricultural ground sulphur	93	90	66	14	6	4	27
Superpoppy sulphur	91	88	53	17	16	2	39
Formaldehyde	85	82	63	7	10	2	23
Germ-O-No	64	62	42	13	3	4	32
Garden Volck	83	80	65	10	4	1	19
Nontreated check	99	95	76	13	4	2	20
Totals	—	882	626	135	93	28	29
			"Schucking" Strain				
Copper carbonate	52	50	44	3	2	1	12
Corona Merko	100	99	72	15	9	3	27
Field's Corn Dust	72	70	56	5	8	1	20
Improved Semesan Jr.	92	89	53	16	17	3	40
Smuttox	34	33	18	8	5	2	45
Agricultural ground sulphur	72	70	35	10	23	2	50
Superpoppy sulphur	100	100	56	26	13	5	44
Formaldehyde	59	57	38	7	9	3	33
Germ-O-No	78	75	32	12	27	4	57
Garden Volck	93	90	39	22	25	4	57
Nontreated check	95	92	57	20	13	2	38
Totals	—	825	500	144	151	30	39

11 per cent showed abnormal vegetatively proliferating ears only. In some instances partly smutted ears and tassels were observed. Wherever smut showed in a tassel, however, all ears on the plant showed smut. The proportionate appearance of diseased tassels and ears and of abnormal ears in the field experiments is distinctly in contrast to the results met with in the greenhouse trials where out of 40 diseased plants only 5 per cent showed smutted ears only, and 43 per cent showed abnormal ears only.

There is evidence to indicate that head smut has been present on corn in Oregon for a good many years. At a meeting of corn growers held in 1931 at the experimental field, a neighboring farmer stated that he had

seen smut in this field as long ago as 35 years. Other fields in this vicinity also showed head smut in 1931. One would expect the disease to be prevalent throughout the Willamette Valley; yet, at a meeting of corn growers held at Oregon City some 40 miles to the north of this experimental field, many in attendance had grown corn for 20 years or more, but none had observed this disease.

SUMMARY

Sorosporium reilianum, head smut of corn, is found destructive in certain localities of western Oregon, while it is absent from others. Experiments were conducted with this disease in the greenhouse and in the field in 1931. The results demonstrated that the disease may be transmitted to a certain extent by seed-borne spores; that the local strain of head smut of corn was not successfully transmitted to 6 varieties of sorghum, whereas it produced abundant infection on 6 varieties of corn; that various seed treatments can be used to prevent seed-borne infection, but that no seed or soil treatment included in these tests was effective in preventing infection in smut-contaminated soil.

OREGON AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

HOW PLANT PATHOLOGY CAN CONTRIBUTE TO A LAND-UTILIZATION PROGRAM

NEIL E. STEVENS

(Accepted for publication July 19, 1932)

The suggestion that an increase in our knowledge of the distribution of plant diseases and their relative severity in different localities is of more immediate economic significance than a further increase in our knowledge of the methods of disease control seems like an indictment of all pathological teaching and present practices. Yet, this is the logic of events, and a plant pathology that neglects economic conditions can lay little claim to being practical. To appreciate how great a change has occurred in our economic thinking it is necessary merely to refer to serious statements of 8 or 9 years ago to the effect that, about 1906, the United States entered a period when population increased faster than agricultural production, that our exportable excess was decreasing, and that within 15 or 20 years our consumption would equal our production. These statements sound like counsels from another civilization.

THE PRESENT PROBLEM

The outstanding features of the present agricultural situation have been widely discussed and there is general agreement as to the need for a planned use of our land resources. The major feature of this program in relation to agricultural land was clearly stated last October by Franklin D. Roosevelt, then Governor of New York, in an article that includes the sentence—"Today in spite of the great increase in our population, the farm acreage problem in the United States is more a problem of reducing our acreage on a sound scientific basis—reducing it where it ought to be reduced—than of expanding it."[1]

PLANT PATHOLOGY AND ACREAGE ADJUSTMENT

That plant-disease information would be important in an adequately planned agricultural adjustment is obvious to the well informed. It is almost equally obvious that for the greatest effectiveness there will be necessary some reorientation of the studies of professional plant pathologists. It may be taken for granted that in any acreage adjustment the "high-cost" acres should be first abandoned. It is evident that a given area may properly belong in the high-cost group because of disease, as well as soil, factors. Any region in which the diseases of a particular crop are severe

[1] Roosevelt, F. D. Planning use of land for farm, forest and highway. Louisiana Conservation Rev. 2 (11): 22–23. Oct., 1932.

or particularly hard to control becomes necessarily a high-cost area for that crop.

In his report to the President for 1931 (pages 37–38) the Secretary of Agriculture urges an economic classification of our land resources and widespread dissemination of information concerning the uses for which particular classes of land are economically adapted on the ground that such a classification would make it easier to discourage ill-advised expansion and lessen the risks of new settlement. A classification of crop areas with reference to severity of plant diseases and the difficulty of their control would have similar usefulness.

The compilation by plant pathologists of a map of the United States showing areas in which disease control on certain crops has proved particularly difficult should be well within our scientific possibilities. Whether it will prove to be a practical possibility may be a quite different matter. It admittedly runs counter to most of our previous practice, as well as to our American psychology. A disease particularly difficult to control offers to the pathologist, of course, a particularly attractive problem. Hitherto, an attempt to grow a crop in a region for which it was not well suited has been accepted by plant pathologists as a challenge to their best efforts.

To suggest the abandonment of a crop project sounds like a council of defeat and is correspondingly repugnant to our temperaments. It is, however, no different essentially from a soil survey that names given areas as being unsuited to profitable cultivation. The suggestion of a nation-wide mapping of areas that, under present conditions, are not worth cultivating sounds like a defeatist policy. It is, however, quite the contrary. The day of expansion of crop production in the United States has definitely passed, perhaps, for a long time. We may well be entering a period of contraction of acreage. Under present economic conditions some lands may even be forced out of production. Obviously, a planned acreage adjustment is preferable to one forced by bankruptcy. And one in which the knowledge of the plant pathologists shall be utilized is better than one planned without reference to plant diseases or to their control.

A few examples of the way in which adequate plant-disease information might have served to "discourage ill-advised and unnecessary expansion" may be cited in the field of small fruits. Information as to the probable difficulty of disease control was already in the hands of plant pathologists, which, if it could have been followed by growers, would have prevented costly and disastrous attempts to grow Vinifera grapes in Texas, Euvitis grapes in Florida and the coastal regions of the Carolinas, raspberries in eastern South Carolina, currants in eastern Maryland, and cranberries in extreme southern New Jersey.

Fortunately, for immediate progress, it is not necessary to plan a crop-loss map to be at once published and widely disseminated. The first steps will be the assembling of available information and the developing of methods of securing better information of this type. It has been frequently pointed out that present economic difficulties offer a challenge to our civilization equal to that of the World War. The present need for classified information regarding plant diseases and their control would appear to offer an opportunity for American plant pathologists second only to that so effectively met by them during the period of the World War.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES

A Hitherto Unreported Blight of the Juglandaceae.—At Stamford, Connecticut, I have a collection of trees. Some of them are employed for hybridizing purposes. In this collection there are various hybrids that have been obtained during the past 35 years.

This year I planned to obtain hybrids between the English walnut, *Juglans regia,* and the butternut, *J. cinerea.* The English walnut was of the Webb variety, which has enormous nuts, and the butternut was one of many seedling natives. In the last week in May I bagged pistillate flowers of the English walnut and then waited for the aments to ripen their pollen and fall from the tree. This would naturally occur at about the time when butternut pollen would be viable. On the morning of the day when the English walnut pollen should have ripened the anthers had all blackened and the pollen was destroyed. Specimens of the ruined aments were sent to the Connecticut Agricultural Experiment Station at New Haven, Connecticut. G. P. Clinton, the Plant Pathologist, expressed the opinion that the blight was bacterial in its nature. A few days later I picked an ament from the butternut tree that was to furnish pollen for the English walnut. In my opinion the butternut would shed pollen at daylight on the following day. The plucked ament was tossed upon the ground. At about 8 o'clock on the following day all of the anthers on the butternut tree were ruined by the blight and were black. The ament that had been tossed upon the ground was shedding its pollen and was not blighted.

During the next few days blight had attacked the anthers of all butternuts, their hybrids, and their parthenogens. It had attacked the anthers of 5 cultivated varieties of the black walnut, *Juglans nigra,* and of several varieties of the Japanese walnut, *J. sieboldii.* No male flowers remained upon *J. sinensis* or upon *J. sibirica.* Unfortunately, I did not examine flowers of a number of species and varieties of the hickories because they did not enter into my hybridization plans for this year; but *Hicoria pecan* aments of 3 varieties were found blighted, and it was observed that *H. ovalis* was the last one of the hickory group to flower and to blight.

Incidentally, some of the butternut aments, which were mostly blighted but bore a little pollen, were carried to the English walnut tree and shaken over pistillate flowers. The appendages of these flowers turned partly or wholly brown the next day. They had not been attacked by blight originally, but the brownish effect seemed to have resulted from contact with blighted aments.

At this date of writing, August 26, many of the walnut trees of several species and varieties that should be heavily laden with nuts have none at

all. Others have a few clusters, as though some pollen had acted very locally on the tree.

The blight seemed to have one notable peculiarity. It apparently did not attack pollen-bearing anthers except on the night when they were ready to shed pollen the following day, and it then destroyed all or nearly all on any given tree on that particular night. Sometimes, the process seemed to be extended over 2 or 3 nights, so that a little pollen escaped from a few flowers or from parts of a few flowers during 1 or 2 days.

During about 35 years of hybridizing experiments I had never previously noticed this blight. If it had been present it was not in sufficient degree to attract attention.—ROBERT T. MORRIS, M.D., Stamford, Connecticut.

A Disorder of Persian (English) Walnuts Grafted on Black-walnut Stocks, Resulting in Girdling.—A disorder of Persian (English) walnuts grafted upon black-walnut rootstock is causing considerable concern to Oregon walnut growers in the Willamette Valley. The trouble was first noted in 1924 in a 15-year-old grafted Franquette orchard near Dundee, Oregon. Since then it has either been found or reported from several other orchards of the Franquette variety in western Oregon.

The first evidence of this trouble is shown by poor shoot growth and by premature shedding of the leaves on a part or all of the tree. As a rule, suckers from the black-walnut rootstock appear at the same time. Death usually follows 3 or more years after the first symptoms are noted in the aerial portions of the tree. The devitalization and death of the tree are apparently caused by girdling brought about by the failure of the newly formed xylem tissues of the Persian top to unite with the black-walnut rootstock. As the new cells of the xylem are laid down by the cambium the two kinds of tissue no longer unite. A dark discolored layer of a corky nature is formed between the two kinds of wood. At first it may be only a fraction of a millimeter in thickness but, in the later stages, it may be as much as $\frac{1}{4}$ in. thick. Only a few inches of the new tissue at the point of union may be affected the first year, but, eventually, the whole circumference of the tree is affected in a like manner. In most cases death of the tree ensues 3 years after the first symptoms are evident. The girdling of the tree is apparently preceded by the presence of water-soaked, chocolate-color spots in the bark of the rootstock at or below the point of union. In the early stages of the trouble these spots are very small and usually isolated. Later, they enlarge to several inches in diameter and often several of these spots merge, forming a large area of dead tissue. In some instances the first visible symptom of the disorder seems to consist of a discolored narrow strip of tissue in the vicinity of the union encircling the tree, but not penetrating to any depth into the bark. In such instances no evidence of a lack of union has been

found by cutting through the bark. In cases where connecting tissue extended from this outer strip into the woody tissue a break in the union always occurred. The trouble has so far been found only on the Franquette variety grafted on a black-walnut rootstock, of which the individual rootstocks are apparently *Juglans hindsi* (Sarg.) (Hinds or Northern California black walnut) or hybrids of other species with this one.

The cause of this disorder is not definitely known. A number of attempts have been made by the junior writer to isolate some parasitic organism from the discolored tissues but to date no infectious microorganism has been found to be constantly associated with the trouble. It appears, therefore, that this disorder is of nonparasitic nature.—C. E. SCHUSTER, Bureau of Plant Industry, United States Department of Agriculture, Corvallis, Oregon; and P. W. MILLER, Division of Horticultural Crops and Diseases, United States Department of Agriculture, Corvallis, Oregon.

THE CANADIAN PHYTOPATHOLOGICAL SOCIETY

Advance Notice of Meeting

Arrangements are being completed for the holding of the 1933 meeting of the Society at Regina, Sask., in July, in conjunction with the World's Grain Fair and the C. S. T. A. Convention. A committee under the chairmanship of Dr. P. M. Simmonds, Saskatoon, has been appointed to look after local arrangements.

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ENVIRONMENTAL FACTORS IN RELATION TO SNAP-BEAN DISEASES OCCURRING IN SHIPMENT

J. I. LAURITZEN, L. L. HARTER, AND W. A. WHITNEY

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INTRODUCTION

Anthracnose (*Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav.), bacterial blight (*Bacterium phaseoli* E.F.S. or *Bact. medicaginis* var. *phaseolicola* (Burk.) Link & Hall), watery soft rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), cottony leak (*Pythium butleri* Subr.), soil rot (*Rhizoctonia solani* Kühn), southern blight (*Sclerotium rolfsii* Sacc.), Rhizopus soft rot (*R. tritici* Saito and *R. nigricans* Ehrnb.), and gray mold rot (*Botrytis cinerea* Pers.) are the principal transit diseases of snap beans, *Phaseolus vulgaris* L.

Some idea of the distribution and relative importance of some of these diseases may be had from the data presented in tables 1 and 2, obtained from market-inspection certificates, issued by the Division of Fruits and Vegetables, Bureau of Agricultural Economics, U. S. Department of Agriculture. The certificates employed represent all the car-lot inspections made at terminal markets in the United States during the years 1922 to 1928, inclusive, and deal with questions of grade and condition, including disease.

Table 1 indicates the States and geographical districts from which the cars originated. It does not, however, show the number of times that a particular disease originated from a particular State or district. It will be seen that watery soft rot is present in all the districts and in most, if not all, of the States. Anthracnose shows the next widest distribution and blight, the third. Some States, mostly along the South Atlantic and Gulf Coast, show the presence of all the diseases.

Table 2 shows that out of 3,107 cars inspected at all the terminal markets in the United States during the years 1922 to 1928, 15 per cent showed watery soft rot; 6.7 per cent, anthracnose; 3 per cent, bacterial blight; 2.3 per cent, soil rot; 1.3 per cent, Rhizopus soft rot; 0.8 per cent, gray mold rot; 3.1 per cent, other diseases (including slimy soft rot and powdery mildew, etc.); 17.1 per cent, injuries (including scarring, insect injury, freezing, etc.); and 50.6, showing no disease. It should be stated that the 15 per cent watery soft rot probably includes some cottony leak that was not distinguished from the former until 1925 (4).

TABLE 1.—*Distribution of the principal transit diseases of beans by States and geographical units*

Unit	State	Anthracnose	Blight	Watery soft rot	Soil rot	Rhizopus soft rot	Slimy soft rot	Gray mold rot
South Atlantic and Gulf Coast	Virginia	+[a]	+	+				+
	North Carolina	+	+	+				
	South Carolina	+	+	+	+	+	+	
	Georgia	+	+	+				+
	Florida	+	+	+	+	+	+	+
	Alabama	+		+		+		+
	Mississippi	+	+	+	+	+	+	+
	Louisiana	+	+	+	+	+	+	+
	Texas	+	+	+	+	+	+	+
Middle Atlantic	New York	+		+				
	New Jersey	+						
	Delaware	+						
	Maryland	+	+	+	+			
South Central East	Kentucky	+						
	Tennessee	+	+	+	+	+		+
South Central West	Oklahoma			+				
	Missouri			+				
	Arkansas	+		+				
North Central	Ohio	+		+	+			
	Michigan			+				
	Illinois			+				
West and Far West	Colorado		+					
	Oregon							
	California			+				

[a] (+) = Presence of disease.

Aside from the work of Ramsey (10) on Sclerotinia soft rot and Harter and Whitney's investigations (4) on cottony leak, the information available on the transit diseases of beans is limited largely to casual observations. Fulton (3) reports that anthracnose probably develops during the marketing of beans on lots that were apparently sound when picked.

Hedgcock (6) described Rhizoctonia lesions on bean pods. Fulton (3) reports damping off of bean seedlings, a dry rot of the stem, and lesions on the pods, all caused by Rhizoctonia. He believes that infection of pods is associated with injury of some kind. He states that the fungus often causes a bronzing in the absence of the development of lesions. Barrus (1) claims that Rhizoctonia attacks only pods lying in contact with the soil.

Ramsey (10), in his extensive studies on some of the species of Sclerotinia causing decay of vegetables, found that, although *S. sclerotiorum*, *S. intermedia* Ramsey, *S. minor* Jagger, and *S. ricini* Godfrey are able to

TABLE 2.—*Relative losses in beans in transit due to diseases and injuries*

Condition	Cars affected		Extremes of decay in cars
	Number	Per cent	Per cent
Watery soft rot	474[a]	15.0	T[d] to 33.0
Anthracnose	207	6.7	T to 38.5
Bacterial blight	93	3.0	T to 75.0
Soil rot	71	2.3	T to 62.0
Rhizopus soft rot	39	1.3	T to 80.0
Gray mold rot	25	0.8	T to 16.0
Other diseases[b]	95	3.1	
Injuries[c]	530	17.1	
No decay	1,573	50.6	

[a] Total number of cars inspected from 1922 to 1928, inclusive—3,107.

[b] Including slimy soft rot, powdery mildew, undetermined soft rot, an unnamed decay, and an unidentified nest.

[c] Including scarring, insect (principally corn-ear worm) injury, freezing, and other uncatalogued blemishes.

[d] T—Trace or less than 1 per cent.

infect and decay beans, *S. sclerotiorum* is responsible for most of the decay occurring in transit. On the losses occurring in transit, he has the following to say (p. 600): "After selecting at random 42 cars of green beans, which on inspection at market showed Sclerotinia decay, it was found that the percentage of infection ranged from 5 per cent in some cars to as high as 75 per cent in others, the average for all the cars being 24 per cent." He obtained infection of slices of turnip and carrots and of lettuce leaves by means of ascospores and of beans by means of mycelium and sclerotia of *S. sclerotiorum*. The range of temperature at which ascospores were germinated in sterile water and nutrient broth was 3–4° to 30–31°.[1] The fungus grew on potato-dextrose agar at temperatures extending from 0° to 32–33° and infected beans from 0° to 27–30°. Harter and Whitney (4) reported the common occurrence of cottony leak during the warmer months of the year in snap beans shipped to northern markets from the South and that the disease probably originated in the field. They compared the decay produced by *Pythium butleri* (*P. aphanidermatum*) with that produced by *S. sclerotiorum, Rhizoctonia solani, Botrytis cinerea, Rhizopus tritici,* and *R. nigricans.* Pythium caused a more rapid decay at warm temperatures (about 25°) than the other pathogens. They (5) found the maximum, minimum, and optimum temperature for growth of *P. butleri* on corn-meal agar to be approximately 38.8°, 6°, and 32°, respectively.

[1] Unless otherwise indicated in the text, the temperatures recorded throughout this paper are expressed in °C.

Important considerations in connection with the transit diseases of beans[2] are source of inoculum, origin of infection (whether in field or in transit), and the effect of temperature and time on infection and the subsequent development of the disease.

In an effort to throw additional light on some of these questions, experiments were conducted during the 6 years 1925 to 1931, at Arlington Experiment Farm, Rosslyn, Virginia.

MATERIALS AND METHODS

Pathogens. The pathogens used in these experiments and their origins are as follows: *Colletotrichum lindemuthianum,* bean; *Bacterium phaseoli,* bean and Lima bean; *Sclerotinia sclerotiorum,* sweet potato; *Pythium butleri,* sweet potato; *Rhizoctonia solani,* bean; *Sclerotium rolfsii,* bean; *Rhizopus tritici,* sweet potato; *R. nigricans,* sweet potato; and *Botrytis cinerea,* pepper. Some experiments were conducted with *C. lindemuthianum* in which the inoculum was obtained from anthracnose lesions on bean pods.

Host. The beans used were obtained on the Washington market and originated in Florida. They were purchased the morning they arrived and used at once in the experiments. The Bountiful variety was mostly used, but each of the varieties Black Valentine, Longfellow, Giant Stringless Green Pod, and an unidentified variety were used once. In all the experiments, defective, abnormal, or infected beans were sorted out.

Technique. The inoculations in case of all the pathogens, except *Colletotrichum lindemuthianum* and *Bacterium phaseoli,* were made in a similar manner. A bit of the fungous growth was placed in a small slit made in the pod, which was then placed in the center of a 4-qt. till basketful of beans. In some of the trials, involving infection by species of Rhizopus, dependence was placed upon these fungi normally present on the beans. In the experiments with anthracnose, spores were either inserted into pods with a needle or sprayed on them with an atomizer. Whenever inoculation experiments were attempted with the blight organism the inoculum was introduced into the bean pods by means of a needle puncture or the beans were atomized with a water suspension of the bacterium. In some of these experiments with the latter pathogen, beans known to be contaminated and possibly infected with blight in the field were used.

Equipment and Control of Temperature and Humidity. The beans were stored either in ventilated insulated chambers (8) or in galvanized iron chambers (9) the temperature of which was controlled by thermoregulators. The humidity of all chambers was governed by water in evaporation pans.

[2] Use of the term "bean" or "beans" throughout this paper signifies snap beans and not shelled beans.

EXPERIMENTAL WORK AND RESULTS

Anthracnose. The ability of *Colletotrichum lindemuthianum* to infect snap beans (Longfellow and Bountiful varieties) has been demonstrated by two types of experiment—one in which spores were inserted into the beans by needle pricks and the other in which the spores from culture or from infected beans were sprayed on the pods with an atomizer. The development of anthracnose lesions has been observed in inoculated sound beans and in inoculated and noninoculated beans contaminated and possibly infected in the field.

Pods inoculated by inserting spores with a needle became infected (Experiment 1, Table 3) within 2 days at temperatures of 15.5°, 19°, 22.5°, 28°, 31°, and 33°. These pods were placed in the center of 4-qt. till baskets of beans (Bountiful variety) and the baskets stored at the various temperatures. At the end of 3 days, infection was evident at 12°. A temperature of 33° is 2° higher than the maximum obtained by Edgerton (2) when the organism was grown on culture media. In a second experiment (Experiment 2, Table 3), infection occurred in 4 days at temperatures ranging from 8° to 31°. Checks were run in both experiments and negative results were obtained at all temperatures.

A quantity of beans (Bountiful variety) was purchased, showing the presence of a slight amount of anthracnose. All beans bearing anthracnose lesions, were carefully culled out and the apparently sound ones were divided into 3 lots. One lot was placed in 4-qt. till baskets and, without further treatment, was stored at various temperatures. The second lot was sprayed with *Colletotrichum lindemuthianum* spores from test-tube cultures grown on bean pods. The third lot was inoculated by spraying with a water suspension of spores obtained from anthracnose lesions on living pods. After inoculation the last 2 lots were transferred to 4-qt. till baskets and stored at the various temperatures with the noninoculated beans (checks).

The results obtained from the checks (Table 4) illustrate the development of anthracnose lesions at various temperatures in beans free from any visible signs of infection at the time they were stored. Lesions developed over a range of temperature from 7.5° to 29°. Lesions had already developed in 6 days at temperatures of 15°, 17.5°, 21°, 25°, and 29°. At temperatures of 12.5°, 10°, and 7.5°, the first lesions were observed after 8, 11, and 18 days, respectively. The lesions may have resulted from incipient infections or new infections or from both.

That lesions may result from the inoculation of beans with spores of anthracnose is evident from the results (Table 4) obtained from the beans inoculated with spores from culture and from live pods, as compared with those obtained from the checks. In most instances the inoculated beans

TABLE 3.—*Relation of temperature to infection of beans (Bountiful variety) inoculated with Colletotrichum lindemuthianum (needle-prick inoculation)*

Experiment 1								Experiment 2				
			Infection after different periods of time in days								Infection in 4 days	
			2		3		7					
Temperature	Relative humidity	Number of inoculations	Infection	Diameter of lesions	Infection	Diameter of lesions	Infection	Temperature	Relative humidity	Number of inoculations	Infection	Diameter of lesions
°C.	*Per ct*		*Per ct*	*Mm.*	*Per ct*	*Mm.*	*Per ct*	°C.	*Per ct*		*Per ct*	*Mm.*
33.0	84	12	100	2.5				35.5	93	30	0[a]	
31.0	92	17	100	2.5				31.0	95	30	60	2.5
28.0	83	14	100	4.0				28.0	80	30	87	3.0
22.5	88	11	91	2.5				23.0	92	30	87	3.0
19.0	89	12	100	2.5				17.5	89	30	93	3.0
15.5	93	11	100	1.0	100	3.5		15.5	94	30	90	2.0
12.0	92	14	0		100	Just started		12.0	95	30	100	2.75
8.0	98	12	0				0	8.0	93	30	53	2.0
6.0	97	11	0				0					

[a] There were lesions in 30 per cent of the punctures at this temperature, but they were abnormal. There was a reddening of all the punctures at this temperature.

TABLE 4.—*Influence of temperature on the development of anthracnose lesions in field-contaminated*[a] *beans (Bountiful variety), noninoculated (artificially), and inoculated with a water spore suspension of Colletotrichum lindemuthianum*

Temperature	Relative humidity	Percentage of infection after different periods of time in days																	
		Inoculated with spores from culture						Inoculated with spores from lesions on bean pods						Noninoculated beans					
		6	8	11	14	16	18	6	8	11	14	16	18	6	8	11	14	16	18
° *C.*	*Per ct.*																		
34.0	91	0.0						0.0						0.0					
29.0	96	0.0						0.0						2.6					
28.0	94	10.0						0.0						0.0					
25.0	93	49.1						65.8						20.0					
21.0	92	34.6						55.6						26.3					
17.5	91	9.8	40.2	67.0				7.0	40.0	94.8				8.3	20.9	35.9	37.2		
15.0	93	7.4	32.0	68.9				3.4	22.3	80.5				6.5	21.6	31.7	36.0		
12.5	94	0.0	0.0	16.2	56.9			0.0	1.8	14.3	67.1			0.0	1.9	8.8	14.0		
10.0	98	0.0	0.0	1.8	12.7			0.0	0.0	0.0	16.7			0.0	0.0	0.7	5.2		
7.5	91	0.0	0.0	0.0	1.8	3.4	7.8	0.0	0.0	0.0	0.9	2.7	6.2	0.0	0.0	0.0	0.0	0.0	0.7
2.0	97	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.9	95	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] A few of the pods showed anthracnose lesions when purchased. These pods were carefully sorted out before starting the experiment.

gave a marked increase in infection over the checks, especially if time enough was allowed for infection resulting from inoculation to take place at any particular temperature under consideration. On the whole, the percentage of infection was higher in beans inoculated with spores taken from the living bean pods than in those inoculated with spores from culture.

Two experiments were run in which beans (Bountiful variety), free or nearly free from field contamination or infection, were inoculated by spraying with a spore suspension of *Colletotrichum lindemuthianum*. One lesion developed at one temperature (31°) in the checks in one of the experiments; the checks in the second experiment showed no evidence of anthracnose. In the first experiment, one lot of beans was inoculated with spores from culture and another lot with spores from the host. In the second experiment spores from the host alone were used. One 4-qt. till basket of inoculated and one of noninoculated beans were stored at different temperatures from 2° to 31° (Table 5). Inspections were made in the

TABLE 5.—*Relation of temperature and time to infection of sound beans (Bountiful variety) inoculated with a spore suspension of Colletotrichum lindemuthianum*[a]

Temperature	Relative humidity	Percentage of beans infected after different periods of time in days													
		3	4	5	6	7	8	9	10	11	12	13	14	15	16
° *C.*	*Per ct.*														
31	93	0	.....	0	.....	0	.....	0							
29	97	0	0	0	0	0	0	0							
27	92	.....	0	4	40	.....	76	.....							
25	95	0	0	28	88	91	100	98							
22	93	0	0	8	64	88	99	98							
17.5	89	0	0	0	0	18	17	72	70	89		96			
15.5	92	0	0	0	0	1	0	23	40	73	76	94	86	97	96
12	93	0	0	0	0	0	0	0.8	0	0	36	25	68	71	84
10	98	0	0	0	0	0	0	0	0	0	2	0	20	20	67
7	92	0	0	0	0	0	0	0	0	0	0	0	0.9	0	0.9
5.5	95	0	0	0	0	0	0	0	0	0	.....	0	.....	0	.....
2	97	0	0	0	0	0	0	0	0	0	0	.....	0	.....	0

[a] The data taken after 3, 5, 7, 9, 12, and 14 days were obtained from one experiment and those for 4, 6, 8, 10, 11, 13, 15, and 16, from a second experiment.

first experiment after 3, 5, 7, 9, 12, 14, and 16 days and in the second after 4, 5, 6, 8, 10, 11, 13, and 15 days. Table 5 contains the composite results of these experiments with snap beans inoculated with a spore suspension from the host, and table 6 shows the results from the one experiment in which beans were inoculated with spores from culture and host. The results given in table 5 are represented graphically in figure 1.

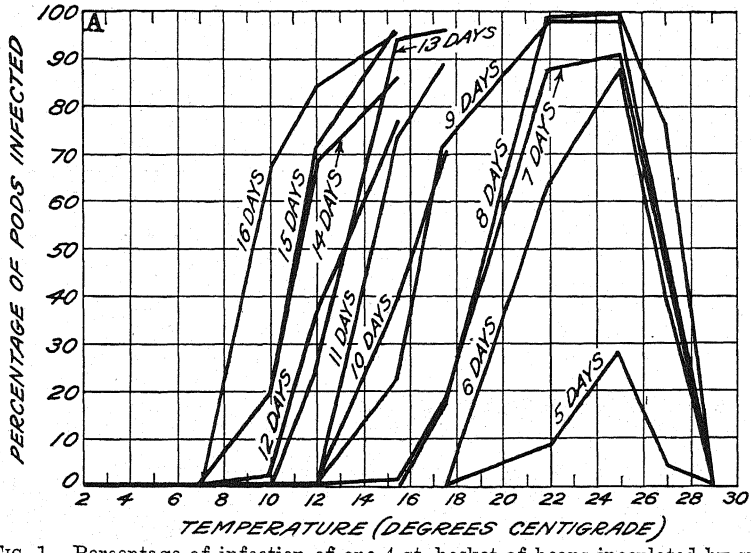


FIG. 1. Percentage of infection of one 4-qt. basket of beans inoculated by spraying with a water spore suspension of *Colletotrichum lindemuthianum* from living bean pods, after different periods of time at various temperatures.

The earliest infection was observed in 5 days. In the first experiment an inspection was not made on the fourth day. However, judged by the amount of infection occurring in 5 days in this particular experiment, there probably was no infection in 4 days, either when inoculated with spores from pure culture or with spores from the host (Table 6). If all the results are taken into consideration, the earliest infections were limited to temperatures of 22°, 25°, and 27°. As the temperature fell below 22°, the earliest infection occurred in 7 days at 15.5° and 17.5°, in 9 days at 12° and 12.5°, in 12 days at 10°, and in 14 days at 7°. No infection occurred at 2° or 5.5° in 16 days. The highest temperature at which infection occurred was 27°, which approximates the maximum (27.2°) (7) obtained for infection of growing bean plants. The lowest temperature at which infection was obtained is 7°, about 7° below the lowest temperature (14.4°) at which infection of growing bean plants was obtained (7). In the latter case the plants were exposed to the various temperatures at humidities favorable to infection for 24 hours, when they were removed to humidities that did not permit infection, whereas, in the former case, the bean pods were stored at favorable humidities at temperatures of 15.5° and below for periods of 11 to 18 days. This difference in time of exposure to conditions favorable to infection, as well as the physiological differences between plants and pods, may account for the difference in the lower temperature limits of infection in the two cases. The optimum temperature

TABLE 6.—*Influence of kind of inoculum on infection of beans (Bountiful variety) by Colletotrichum lindemuthianum at different temperatures*

Temperature	Relative humidity	Percentage of infection after different periods of time in days													
		3		5		7		9		12		14		16	
		Culture[a]	Host	Culture	Host	Culture	Host	Culture	Host	Culture	Host	Culture	Host	Culture	Host
° C.	*Per ct.*														
31	93	0	0	0	0	0	0	0	0						
29	97	0	0	0	0	0	0	0	0						
25	94	0	0	0.9	7	32	91	43	98						
22	93	0	0	0.8	3	58	88	69	98						
17.5	91	0	0	0	0	8	18	22	72						
15.5	90	0	0	0	0	0	1	10	23	42	76	43	86	50	96
12.5	93	0	0	0	0	0	0	0	0.8	20	36	58	68	70	83
10	98	0	0	0	0	0	0	0	0	0.8	2	13	67	65	67
7	91	0	0	0	0	0	0	0	0	0	0	0	0.9	0	0.9
2	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] In the one instance the beans were inoculated with a spore suspension from pure culture on sterile bean pods and in the other with a spore suspension from lesions on live beans.

for infection of the pods ranges from 22° to 25°, the highest percentage of infection usually occurring at 25°. The optimum temperature for growth of Colletotrichum on culture media was 21° to 23° (2).

It will be noted (Table 6) that the percentage of infection at any given temperature and period of time was higher in beans inoculated with spores from the living pods than from the culture. Infection was also obtained at a lower temperature (7° as compared with 10°) in the former than in the latter case. This fact, taken in connection with similar results given in table 4, suggests that spores taken directly from the host may be more pathogenic than spores from artificial culture media, or there may be a loss of virulence in culture. It may, however, merely mean that a heavier spore suspension was used in the one case than in the other.

Bacterial Blight. *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola* cause lesions so similar on bean pods that they are not easily distinguishable, and no attempt has been made in these investigations to differentiate between lesions produced by them. *Bacterium phaseoli* alone has been used in the inoculation experiments.

The ability of *Bacterium phaseoli* to infect snap-bean pods detached from vines was tested in two ways: through wounds and by spraying with a water suspension of the pathogen.

Two experiments were conducted in which the needle-prick method was used, and infection (Table 7) took place readily over a range of tempera-

TABLE 7.—*Relation of temperature to infection of beans*[a] *by Bacterium phaseoli (needle punctures)*

Experiment 1											Experiment 2										
			Infection and decay after different periods of time in days											Infection and decay after different periods of time in days							
			4		6		10		15					4		6		8		11	
Temperature	Relative humidity	Number of inoculations	Infection	Diameter of lesions	Infection	Diameter of lesions	Infection	Diameter of lesions	Infection	Diameter of lesions	Temperature	Relative humidity	Number of inoculations	Infection	Diameter of lesions	Infection	Diameter of lesions	Infection	Diameter of lesions	Infection	Diameter of lesions
	Per		*Per*		*Per*		*Per*		*Per*			*Per*		*Per*		*Per*		*Per*		*Per*	
°C.	*cent*		*cent*	*Mm.*	*cent*	*Mm.*	*cent*	*Mm.*	*cent*	*Mm.*	*°C.*	*cent*		*cent*	*Mm.*	*cent*	*Mm.*	*cent*	*Mm.*	*cent*	*Mm.*
35.5	95	15	0								35.5	93	11	0	0						
31	91	15	93	4							31	95	28	64	25						
24	95	14	79	2.5							24.5	94	33	88	2.25						
21.5	91	15	0	0	66	3	100	4.5			21	91	30	7	–						
15.5	93	15	0	0	0	0	27	3			15.5	94	32	0	0	25	3.25				
12	90	15	0	0	0	0	0	0	0[b]	0	12	95	31	0	0	0	0	0	0		
8	93	15	0	0	0	0	0	0	7	–	8	93	30	0	0	0	0	0	0	10	4.5
6	95	15	0	0	0	0	0	0	0	0	2	98	32	0	0	0	0	0	0	56	1.25
2	97	15	0	0	0	0	0	0	27	1											

[a] The Black Valentine variety was used in Experiment 1; the Bountiful variety in Experiment 2.

[b] Pods partially decayed by Botrytis.

ture extending from 2° to 31°. No infection occurred at 35.5°. Considerable infection had occurred at temperatures of 24°, 24.5°, and 31° in 4 days in the two experiments, and in one of the experiments 7 per cent of the inoculations at 21° resulted in infection in 4 days. In one experiment at 15.5° there was 25 per cent infection in 6 days and, in the other, 27 per cent in 10 days. In one experiment there was no infection in 8 days at 12° and none in 15 days in the other. In the latter case the results were complicated by infection by *Botrytis cinerea*. Infection was evident in 11 days at 8° and 2° in one experiment and in the other experiment, at 2°, but not at 6° in 15 days.

Three experiments were conducted in which a water suspension of *Bacterium phaseoli* was sprayed on snap-bean pods by means of an atomizer. In the first 2 experiments, blight was present on the beans when purchased. In the third it was absent. In the former two all the infected pods were sorted out before inoculating the remaining beans. In each case the beans were stored at various temperatures in 4-qt. till baskets. The temperature range employed for each of the 3 experiments was as follows: 5.5° to 29° (11 temperatures), 2° to 31° (10 temperatures), and 1.2° to 29° (11 temperatures), respectively. The relative humidities were above 90 per cent in all cases. All 3 experiments yielded negative results so far as the effects of inoculation are concerned. This fact is not so clear in those experiments in which blight infection was present in the original stock as it is in the one in which blight was absent. The results of one of these experiments in which blight was originally present are given in table 8. The findings of the second experiment were similar. There was even slightly less infection in the inoculated as compared with noninoculated beans (Table 8), as indicated by the compensated percentages that represent the difference between the percentage of infection in the checks and the inoculated beans. The absence of infection was not due to a lack of virulence on the part of the pathogen because infection resulted in beans inoculated by puncturing the pods with a needle contaminated with the organism and stored at the various temperatures along with the sprayed beans. Seventy-two per cent of the 25 needle-prick inoculations at 28° resulted in infection in 3 days, 56 per cent at 21° in 11 days, 8 per cent at 17° in 11 days, and 24 per cent at 15° in 11 days. No checks were run in connection with the other 2 experiments, but the needle-prick method of inoculation has always yielded positive results in our experiments.

The results obtained from inoculations with a bacterial suspension may be interpreted in one of two ways: either the conditions suitable for infection were not met or *Bacterium phaseoli* normally lacks the ability to infect uninjured pods detached from the vines.

TABLE 8.—*Development of blight at different temperatures in beans (Bountiful variety) inoculated with a water suspension of Bacterium phaseoli*

Temperature	Relative humidity	Beans used	Inoculated beans: Percentage of beans infected after different periods of time in days						Beans used	Noninoculated beans (checks): Percentage of beans infected after different periods of time in days						Compensated percentages: Differences in percentage of infection in inoculated and noninoculated beans after different periods of time in days					
			6	8	11	14	16	18		6	8	11	14	16	18	6	8	11	14	16	18
° *C.*	*Per ct.*	*Number*							*Number*												
29	94	141	14.2						90	6.7						+7.5					
28	96	121	3.3						152	6.6						−3.3					
25	93	146	14.4						99	16.2						−1.8					
21	92	152	7.2						147	12.9						−5.7					
17	91	138	4.4	37.0	32.0	38.4			148	5.4	22.3	49.3	53.2			−1.0	+14.7	−17.3	−14.8		
15	93	144	4.9	20.8	29.2	41			139	5.8	15.2	40.3	43.9			−0.9	+ 5.6	−11.1	− 2.9		
12	94	117		10.3	19.7	24.8			159		9.4	14.6	26.4				+ 0.9	+ 5.1	− 1.6		
10	98	136			3.7	11			135			5.9	10.4					− 2.2	+ 0.6		
7	91	121				5	11.6	12.4	135				10.4	15.6	13.3				− 5.4	−4.0	− 0.9
2	97	116					12.9	23.3	145					18.6	33.8					−5.7	−10.5
1.2	95	112					16.1	36.6	150					22	44.7					−5.9	− 8.1

The results submitted in table 8, as well as those obtained from the other experiment in which blight was found present in the original stock, indicate that blight may develop in snap beans stored at various temperatures in which there were no visible signs of the disease at the beginning of storage. Blight lesions developed at temperatures from 1.2° to 29°. After 6 days' storage infection in noninoculated beans varied from 5.4 to 16.2 per cent at temperatures from 15° to 29°. There was an increase in the percentage of infection with the lapse of time at temperatures below 21°.

In order to give a more adequate picture of the development of blight as related to temperature and time, daily readings were taken of the blight present in beans of the Bountiful and Giant Stringless Green Pod varieties stored at 11 different temperatures in 4-qt. till baskets. About 7 per cent of the pods of both varieties were infected when purchased on the market. They had been 3 days *en route* from Canal Point, Florida, to Washington, D. C. The blighted pods were carefully sorted out and the rest of the beans thoroughly mixed to insure a fairly uniform distribution of contaminated beans or those that may have been carrying incipient, invisible infections.

The results obtained from the 2 varieties were so similar that only those of the Giant Stringless Green Pod will be presented (Table 9). Blight developed at all temperatures, although very little at 35°, indicating that this temperature is approaching the maximum. There is a wide range (10°

TABLE 9.—*Influence of temperature and time on the development of blight in beans of the variety Giant Stringless Green Pod*

Temperature	Relative humidity	Pods used	Percentage of beans infected after different periods of time in days								
			1	2	3	4	5	6	7	8	11
° C.	*Per cent*	*Number*									
35.0	92	288	0.3	0.3	0.3	0.3	0.3	0.3	0.3		
31.0	92	224	0.9	1.4	2.2	2.7	4.0	6.7	8.9		
28.5	95	260	1.2	3.5	5.4	8.1	8.1	9.5	8.8		
24.5	91	233	4.3	6.8	9.4	9.0	11.2	13.3	12.9	14.2	
24.5	91	249	2.0	5.2	6.4	6.4	8.0	8.0	11.2	15.3	
19.5	94	298	5.0	7.1	6.7	8.4	8.4	9.1	9.1	13.1	
19.5	94	274	6.2	7.7	9.1	11.0	10.2	13.1	11.7	11.3	
17.5	91	244	4.9	4.1	5.7	9.0	8.6	11.1	11.9	12.3	
15.0	94	286	3.2	4.5	5.2	8.4	11.2	12.6	12.6	12.6	
13.0	93	243	1.7	3.3	4.9	4.9	8.2	9.1	8.2	9.1	
10.0	99	268	1.9	3.0	3.7	4.9	9.3	9.3	11.6	11.9	
7.0	93	234	1.3	2.6	2.6	2.1	2.6	5.1	5.6	6.4	7.2
2.0	97	263	.....	2.5	2.5	2.7	4.6	4.2	4.2	4.2	4.2

to 24.5°) of temperature at which there was little difference in the percentage of pods infected. There is some irregularity in the percentage of infection within this range, persisting at certain temperatures throughout the duration of storage (compare results at 10°, 13°, and 15°). As the temperature fell below 10° the percentage of infection generally decreased. In the case of the variety Bountiful there was a gradual decrease as the temperature fell below 17.5°.

New infections may not normally occur in pods detached from the vines, but blight will develop over a wide range of temperature in contaminated lots sorted free of any visible signs of infection.

Watery Soft Rot. Infection and the spread of watery soft rot (*Sclerotinia sclerotiorum*) during transit are probably primarily effected by means of mycelium and possibly in some instances by ascospores and sclerotia. Its development probably results from the inclusion of infected beans and possibly of ascospores and sclerotia in the shipping package. The mycelium radiates from local points of infection and soon forms a continuous mass of decayed beans and mycelium, known in the trade as a "nest."

The rate of development of these nests, at various temperatures, is an important element in determining the losses occasioned during transit.

A series of 5 experiments was conducted from 1926 to 1928, with 3 varieties of beans (Bountiful in 3 experiments, Longfellow in 1, and an unidentified variety in 1. The results of 2 experiments are presented in tabulated form in table 10. The comparative rates of decay in the 5 experiments at various temperatures after a given period of time are represented graphically in figure 2, A. Infection was obtained within a temperature range of 0° to 28°. Infection occurred in 1 experiment at 27.5° in 5 days (Table 10) and in a second at 28° in 7 days. No infection developed in a third in 4 days at 28° or in a fourth in 5 days at 28.5°. A temperature of 31° was employed in 1 experiment and a temperature of 32° in 2 experiments. There was no infection in the former in 4 days nor in the latter in 5 and 7 days, respectively. These temperature limits for infection of snap beans correspond almost exactly with those (0° to 27–28°) obtained by Ramsey (10).

Infection occurs very slowly at 0° to 2°. In 2 experiments in which the duration of storage was 20 days at 2°, the number of pods infected besides the inoculated bean was 1 and 2, respectively. In 2 experiments infection was limited to the inoculated beans after 15 days' storage at 1.8°. In a third experiment 1 pod, besides the inoculated bean, became infected in 15 days at 1.8°. Infection was not observed in a shorter time at this temperature. Infection occurred in 9 days at 5.5° to 6°, in 7 days at 8° (data not given in table), and in 4 days at 12° (Table 10). The maximum number of infections occurred at temperatures ranging from 19° to 24.3° in 4 of

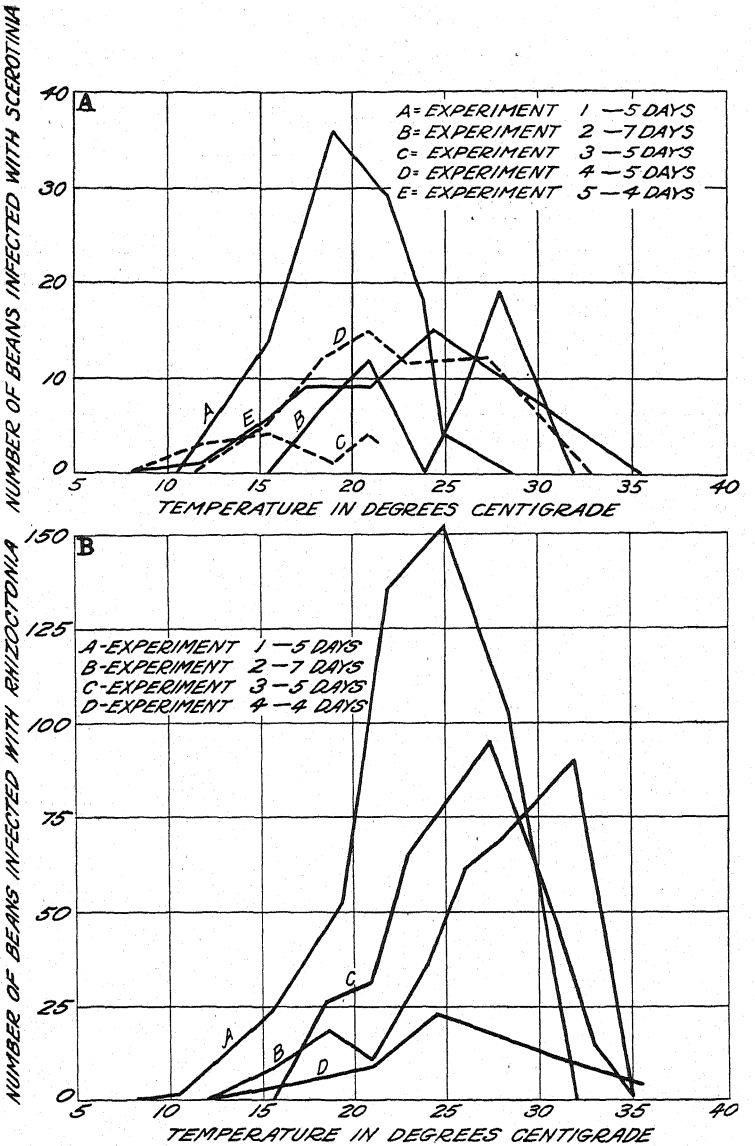


FIG. 2. Relation of temperature to bean infection after different periods. A. *Sclerotinia sclerotiorum*. B. *Rhizoctonia solani*.

the 5 experiments. In the fifth experiment the largest number of infections occurred at 28° (Fig. 2, A). It is believed that this temperature is above the optimum, thought to lie between 19° and 24°. This fact is indicated from the average optimum obtained from the 3 experiments in which

TABLE 10.—*Relation of temperature and time to infection of snap-beans*[a] *by Sclerotina sclerotiorum*

Experiment 1										Experiment 2											
Infection and decay after different periods of time in days										Infection and decay after different periods of time in days											
		5		11		15		16				4		6		8		11		15	
Temperature	Relative humidity	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Temperature	Relative humidity	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest
° *C.*	*Per cent*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	° *C.*	*Per cent*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*
35.2	89	0	0							35.5	93	0	0								
33	84	0	0							24.5	94	15	46								
27.5	83	12	65							21	91	9	41								
23	88	12	82							17.5	89	9	34								
21	90	15	82							15.5	94	6	28	21	48						
18.5	89	12	70							12	95	1	.....	6	35						
15.5	93	5	32	71	122	33	82	163	140	8	93	0	0	5	23	2	20	6	33		
11.5	92	0	0	27	50	125	125	125	140	2	98	0	0	0	0	0	0	1[b]	.....	1[b]	.....
7.5	98	0	.....	3	27	41	82	50	100	0	97	0	0	0	0	.....	.....	0	0	1[b]	.....
6	98	0	.....	1	20	14	52	10	50												
2	97	0	0	0	0	1	18	1[b]	.....												

[a] An unidentified variety was used in Experiment 1 and the Longfellow variety in Experiment 2.
[b] Only the inoculated bean was infected.

inspection was made in 5 days (Fig. 2, A). The lack of uniformity in the results of the different experiments at a given temperature is explained, in part, by the fact that the results of individual inoculations are expected to vary in the amount of decay they produce.

Cottony Leak. The nesting of beans, caused by *Pythium butleri* (*aphanidermatum*), is very similar (4) to that produced by *Sclerotinia sclerotiorum* and, before 1927, the decays produced by these organisms were not differentiated. Although it is not definitely known just how decay is initiated in transit, it is believed to be due largely to the inclusion of infected pods in the hampers of beans at the time of picking. Healthy beans become infected if placed in contact with diseased pods. As a result, nests, sometimes involving several quarts of beans, are formed in the containers during the shipping period.

In order to study infection and the development of cottony leak at various temperatures, 3 tests (only 1 reported in tabulated form, Table 11) were made in which the beans (Bountiful, Longfellow, and an unidentified variety) were inoculated and stored in the same fashion as in the experiments with watery soft rot. Infection occurred at 12° to 35.6°. Higher temperatures than 35.6° were not employed. Infection was limited to the inoculated bean at 12° and 15.5°, even after 16 days' storage. This temperature is 6° above that of the minimum growth of the pathogen in pure culture, as obtained by Harter and Whitney (5). The maximum amount of decay was obtained at 31°, above and below which decay decreased as the temperature was raised or lowered. The shortest time required for infection to occur at the higher temperatures was not determined. Judging by the amount present in 2 days at temperatures from 21° to 35°, its development is very rapid.

Soil Rot. Field infection of bean pods probably originates in most, if not all, instances from contact of the pods with the soil, although it is conceivable that it may occur in other ways, such as from the splashing of infested soil onto the pods or vines during heavy rains. Infection of beans in transit by *Rhizoctonia solani* may result in nesting. As a rule, however, the fungus grows superficially through a lot of beans, infecting pods at local points and forming definite concentric lesions on the pods.

A series of 5 experiments was conducted from 1926–1928 in which 3 varieties of beans (Bountiful in 3 experiments, Black Valentine and an unidentified variety in each of the other 2) were used. The beans were inoculated and stored at various temperatures in the same manner as in the experiments with watery soft rot. The results of 3 experiments are given in table 12. The highest and lowest temperatures at which infection was obtained were 35.5° and 0.9°, respectively. Only in 1 experiment was infection observed at temperatures below 11.5°. The first evidence of in-

TABLE 11.—*Relation of temperature and time to infection of beans (unidentified variety) by Pythium butleri*

Temperature	Relative humidity	Infection and decay after different periods of time in days									
		2		3		5		11		16	
		Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected
°C.	*Per cent*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*
35.0	89	35	62	50	118						
33.0	84	31	53	48	136						
31.0	92	34	62	55	150						
28.0	83	26	26	35	60						
22.0	88	19	17	28	36						
21.0	90	8	5	7	4						
18.5	89	0	0	7	4						
15.5	93	0	0	0	0	–	1	5	1	–	1
12.0	92	0	0	0	0	0	0	–	1	–	1
7.5	98	0	0	0	0	0	0	0	0	0	0
5.5	98	0	0	0	0	0	0	0	0	0	0
2.0	97	0	0	0	0	0	0	0	0	0	0

TABLE 12.—*Relation of time and temperature to infection of beans*[a] *by Rhizoctonia solani*

Experiment 1					Experiment 2					Experiment 3						
Temperature	Relative humidity	Beans infected after indicated time (days)			Temperature	Relative humidity	Beans infected after indicated time (days)			Temperature	Relative humidity	Beans infected after indicated time (days)				
		5	16	20			5	11	16			5	6	8	11	15
° C.	*Per cent*	*Number*	*Number*	*Number*	*° C.*	*Per cent*	*Number*	*Number*	*Number*	*° C.*	*Per cent*	*Number*	*Number*	*Number*	*Number*	*Number*
32.0	92	0			35.5	89	1			35.5	93	4				
28.5	88	103			33.0	84	15			31.0	95	11				
25.0	97	152			31.0	92	46			24.5	94	23				
22.0	91	135			27.5	83	95			21.0	91	9				
19.0	90	53			23.0	88	65			15.5	94	3	1			
15.5	88	24			21.0	90	32			12.0	95	0	0	0	0	
10.5	93	2	3		18.5	89	27			8.0	93	0	0	0	0	0
8.0	98	0	1[b]		15.5	93	0	0	273	6.0	98	0	0	0	0	0
5.5	98	0	1[b]		11.5	92	0	0	4	2.0	97	0	0	0	0	0
2.0	97	0	0	1[b]	7.5	98	0	0	0							
0.9	97	0	0	1[b]	6.0	98	0	0	0							
					2.0	96	0	0	0							

[a] The variety Bountiful was used in Experiments 1 and 2 and an unidentified variety in Experiment 3. The number of infections at each temperature and period of time resulted from a single inoculation.

[b] Infection confined to inoculated pod.

fection in the inoculated pod at temperatures of 0.9° and 2° occurred in 20 days, and in 16 days at 5.5° and 8° (Experiment no. 1, Table 12). A temperature of 35° to 35.5° was employed in 4 out of the 5 experiments. Infection was obtained at this temperature in only 2 experiments. In the fifth experiment there was infection at 32°, the highest temperature employed. It would seem from these results that a temperature of 35° is near the maximum for infection. There was some variation in the temperature at which the maximum amount of infection (Table 12 and Fig. 2, B) occurred. The number of inoculations at a given temperature was here also a limiting factor. Furthermore, it is believed that a normal variation in a temperature optimum is to be expected. The optima obtained in all 5 experiments lie within the limits of 24° and 32°, respectively. Considerable infection may take place at 15.5° to 33°. Although infection has occurred in 5 days at 10.5° (Experiment 1, Table 12), it is usually very limited below 15.5°, even after 15 to 20 days' storage. The rate of development of the number of beans showing Rhizoctonia lesions between 20° and 30° is high. (Fig. 2, B.)

Southern Blight. Infection of beans by *Sclerotium rolfsii* during transit probably results from the inclusion in the pack of soil-contaminated bean pods in much the same way as in the case of *Sclerotina sclerotiorum* and *Rhizoctonia solani.* Nesting occurs in the same manner as it does in watery soft rot and cottony leak.

Three experiments (only 1 reported in tabulated form, Table 13) similar to those of watery soft rot were conducted with *Sclerotium rolfsii.* Infections were obtained at temperatures from 8° to 35.5° (Table 13). Judging by the number of pods (7 in 1 and 3 in another experiment) infected in 3 days at 35.6° in 11 days at 8° infection was limited to the mere beginnings of decay of the inoculated pod. No infection occurred at 6° in the 3 trials. The maximum number of infections in each trial in 3 and 4 days occurred at 31° (Fig. 3). As the temperature rose above or fell below 31°, the rate of decay declined rapidly. Infection had occurred at temperatures of 17.5°, 23°, 28°, 31°, and 35.5° in 2 days. Decay had just started at 17.5°. The earliest infection (inoculated pod) at 12° occurred in 5 days (data not included in table) and at 8° in 11 days (Table 13).

Rhizopus Soft Rot. Although no accurate survey has been made of the species of Rhizopus responsible for decay of beans in transit, *R. tritici* and *R. nigricans* are the only ones that have been noted. The species are sufficiently omnipresent to be a source of decay in beans in transit whenever the conditions are favorable for infection. Nesting is a characteristic of Rhizopus decay, although it is not so pronounced as in watery soft rot, cottony leak, and southern blight.

TABLE 13.—*Relation of temperature and time to infection and decay of bean pods (Longfellow variety) by Sclerotium rolfsii*

Temperature	Relative humidity	Infection and decay after different periods of time in days											
		2		3		6		8		11		15	
		Diameter of nest	Beans[a] infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected
° C.	*Per cent*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*
35.5	93	40	10	33	7								
31.0	95	38	11	90	53								
28.0	80	48	10	78	23								
23.0	92	28	4	63	25								
17.5	89	—	1	8	2	53	15						
12.0	95	0	0	0	0	–	1	–	1	9	1		
8.0	93	0	0	0	0	0	–	0	0	–	1		
6.0	98	0	0	0	0	0	0	0	0	0	0	0	0

[a] The beans infected at each temperature and after each period of time resulted from a single inoculation at the center of a till basketful of beans.

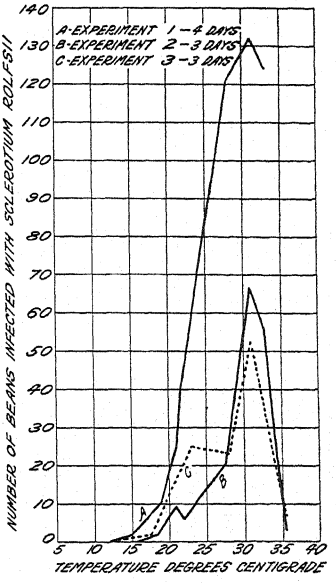


FIG. 3. Relation of temperature to the rate of decay of beans by *Sclerotium rolfsii* at different temperatures in 3 or 4 days in three experiments.

Two experiments were run with each of *Rhizopus tritici* and *R. nigricans* in which the beans were inoculated in the same manner as in the case of watery soft rot and stored at the various temperatures in 4-qt. till baskets. The diameter of the nest and the number of beans infected about a point of inoculation were used as criteria of decay. Data also were taken in connection with noninoculated beans (checks) stored at the various temperatures in the experiments dealing with the other diseases. The results of one of the experiments with inoculated beans are given in table 14 and those of the noninoculated beans in table 15.

There was little difference in the amount of infection or decay between bean pods inoculated with *Rhizopus tritici* and *R. nigricans* in either experiment. At 12° and 15.5°, inoculation with *R. tritici* resulted in infection in 1 experiment, while inoculation with *R. nigricans* caused decay in both. Inoculation of beans by either species resulted in heavier and more uniform infection than is the rule in beans stored without inoculation. The inclusion of an infected bean in the container at the time of shipment, therefore, would be expected to aggravate greatly the losses incurred during transit at temperatures favorable to infection and decay.

Infection has been obtained in noninoculated and inoculated beans over a range of 12° to 35.5°. No infection ever has been obtained at temperature below 12°, even after 16 to 20 days' storage, whether the beans were

TABLE 14.—*Relation of temperature to infection and decay of beans (Bountiful variety) by Rhizopus tritici and R. nigricans*

Temperature	Relative humidity	*Rhizopus tritici*						*Rhizopus nigricans*					
		Infection and decay after different periods of time in days											
		4		5		7	9	4		5		7	9
		Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Beans infected	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Beans infected
°C.	*Per cent*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Number*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Number*
33.0	84	43	100					29	120				
31.0	92	40	110					22	65				
28.0	83	8	90					14	100				
21.5	88	0	0	3	35			1	25				
19.0	89	0	0	1	—			0	0	3	45		
15.5	93	0	0	1	—			0	0	1	—		
12.0	92	0	0	1	10			0	0	1	—		
8.0	98	0	0	0	0	0	0	0	0	0	0	0	0
6.0	98	0	0	0	0	0	0	0	0	0	0	0	0
2.0	97	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 15.—*Normal infection by species of Rhizopus in 5 lots of beans*[a]

Lot 1			Lot 2			Lot 3			Lot 4			Lot 5		
Temperature	Beans infected by Rhizopus	Storage period	Temperature	Beans infected by Rhizopus	Storage period	Temperature	Beans infected by Rhizopus	Storage period	Temperature	Beans infected by Rhizopus	Storage period	Temperature	Beans infected by Rhizopus	Storage period
°C.	*Number*	*Days*	°C.	*Number*	*Days*	°C.	*Number*	*Days*	°C.	*Number*	*Days*	°C.	*Number*	*Days*
35.5	3	4	35.5	9	4	33.0	4	4	35.2	Few	5	32.0	Many	5
31.0	0	4	31.0	0	4	31.0	8	4	33.0	1	5	29.0	5	5
28.0	3	4	28.0	0	4	28.0	0	4	31.0	3	5	25.0	5	5
24.5	6	4	24.5	0	4	21.5	0	5	28.0	0	5	23.5	0	5
23.0	2	4	23.0	0	4	19.0	0	5	23.0	0	5	22.0	0	5
21.0	0	6	21.0	0	6	15.5	0	5	21.0	1	5	19.0	0	5
17.5	8	6	17.5	0	6	12.0	0	5	18.5	0	5	15.5	0	5
15.5	3	6	15.5	1	6	8.0	0	7	15.5	Scattered infection	17	10.5	0	16
12.0	4	11	12.0	1	11	6.0	0	7	12.0	"	17	8.0	0	16
8.0	0	11	8.0	0	11	2.0	0	7	8.0	0	17	5.5	0	16
6.0	0	15	6.0	0	15				6.0	0	17	2.0	0	20
2.0	0	15	2.0	0	15				2.0	0	17			
0.0	0	15	0.0	0	15									

[a] The varieties of beans used were as follows: Lot 1, Longfellow; Lots 2, 3 and 5, Bountiful; Lot 4, an unidentified variety.

inoculated or not. Although the normal occurrence of Rhizopus infection at the various temperatures is somewhat erratic, it occurs commonly at temperature above 25° and sometimes in large amounts, especially if the temperature goes above 30°.

Gray mold rot. *Botrytis cinerea* is more or less omnipresent, and infection of beans may occur in transit whenever conditions of temperature and moisture are favorable. Infection may begin in the field through contact of the pods with the soil. Because of the slow development of Botrytis, the losses in transit are rarely ever large.

The study of the effect of temperature on infection and decay by *Botrytis cinerea* involved 3 experiments in which a snap bean was inoculated by placing a small bit of the fungus and agar in a slit made obliquely in the pod by means of a sterile knife. The inoculated pod was then placed at the center of a 4-qt. till basket of beans. In 2 of the experiments the quantity of beans at each temperature was limited to 1 basket and in the third to 2 and 3. The results given in table 16 are from the latter experiment. The variety Bountiful was used in each experiment.

Infection was obtained at a range of temperature from 0° to 35.5°. In 4 days some infection had taken place at 12°, 15.5°, 17.5°, 23°, 28°, and 35.5°. Judging by the number of infections at the various temperatures,

TABLE 16.—*Relation of temperature to infection of beans (Bountiful) by Botrytis cinerea*

Temperature	Relative humidity	Infection and decay after different periods of time in days									
		4[a]		6		8		11		15	
		Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected	Diameter of nest	Beans infected
°C.	*Per cent*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*	*Mm.*	*Number*
35.5	93	11	1.0								
28.0	95	9	0.5								
23.0	92	17	2.0								
17.5	89	21	3.5								
15.5	94	.....	2.5	38	10						
12.0	95	.....	1.0	20	3	45	10				
8.0	93	0	0.0	9	1	18	2	38	7		
8.0	98	0	0.0	9	3	.....	1[b]	30	2	45	10
2.0	96	0	0.0	0	0	0	0	.....	1	20	2
0.0	90	0	0.0	0	0	0	0	0	0	9	1

[a] The results on the fourth day or days are the averages of 2 baskets of beans at each temperature, those for the remaining period from 1 basket.

[b] The results at 6° were obtained from 3 baskets, after 6 days from 1, after 8 and 11 days from a second, and after 15 days from a third.

decay was mostly in the initial stages. In another experiment infection occurred at 22.5° in 2 days at at 6° and 8° in 6 days. The first infections observed at 2° occurred in 11 days and at 0° in 15 days. The maximum number of infections occurred at 17.5° in 4 days. In another experiment infection had occurred at 22.5° in 2 days, but none at the other temperatures. In the absence of more comprehensive results the optimum may be tentatively placed somewhere between 15.5° and 23°.

DETERIORATION OF SNAP BEANS NOT CAUSED BY DISEASE

A consideration of the conditions affecting development of transit diseases would be incomplete without some discussion of the effect of these conditions on the beans themselves.

Various signs of deterioration, such as bleaching, yellowing, rusting or bronzing, loss of turgor, and a consequent tendency to toughness, may develop during storage. These signs of deterioration lack sufficient definiteness to indicate their development very accurately. An effort, however, was made to characterize the loss of quality in the checks run parallel to the disease tests. (Tables 17 and 18).

In considering the results it should be kept in mind that the beans were from 4 days to possibly a week old when they were stored. It required from 2 to 4 days to transport the beans from Florida to Washington. The time between picking and loading undoubtedly varied, and the temperature and humidity in the railroad car during transit are unknown. It can be said, however, that they were sufficiently favorable to permit them to arrive in Washington in good condition.

The deterioration was rather rapid at the higher temperatures, taking place the most rapidly at 35° and 35.5°, the highest temperature employed, and decreasing in rate as the temperature was lowered. At 6° to 7° they retain their quality from 4 to 15 days. Below 6° the quality remained good from 6 to 15 days.

DISCUSSION AND CONCLUSIONS

In addition to the effects of temperature and humidity on infection and development of disease in beans *en route* to the market, important differences in diseases and in the pathogens that cause them must be taken into consideration in connection with any program dealing with the reduction of the losses in transit and marketing. Anthracnose and blight differ from the other diseases in being seed-borne, which involves the necessity of growing beans from seed free from these diseases. *Colletotrichum lindemuthianum, Pythium butleri, Rhizopus tritici, R. nigricans,* and *Botrytis cinerea,* reproduced by asexual spores can spread readily and rapidly. The asexual spores of the Rhizopus species and *B. cinerea* probably are spread more

TABLE 17.—*Deterioration of snap beans not caused by disease in various lots stored at different temperatures*

Experiment 1[a]				Experiment 2[b]				Experiment 3[c]			Experiment 4[d]			Experiment 5[e]				
Temperature	Relative humidity	Duration of storage	Color and condition	Temperature	Relative humidity	Duration of storage	Color and condition	Temperature	Relative humidity	Condition of beans after 3 days' storage	Temperature	Relative humidity	Condition of beans after 7 days' storage	Temperature	Relative humidity	Condition of beans after different periods of storage in days		
																4[f]	6	8
°C.	*Per cent*	*Days*		°C.	*Per cent*	*Days*		°C.	*Per cent*		°C.	*Per cent*		°C.	*Per cent*			
35.0	89	5	Yellow	35.5	93	4	Ripening and yellowing	33.6	91	Yellow to brown, very little snap								
33.0	84	5	"	31.0	95	4	"	29.0	96	Yellowing, very little snap	31.0	93	Yellow, maturing rapidly					
31.0	92	5	"	28.0	80	4	"	28.5	94	Slight yellowing, very little snap	29.0	97	Yellowish green to yellow	29.0	97	Yellowing	Yellowing	
28.0	83	5	"	24.5	94	4	"	25.0	93	Very slight yellowing very slight snap	25.0	94	Yellowish green	27.0	92	"	"	
23.0	88	5	Beginning to yellow	23.0	92	4	"	21.0	92	Color good as at start, snap slight	22.0	93	Pale green	25.0	95	"	"	
21.0	90	5	"	21.0	91	6	"	17.5	91	"	17.5	91	" "	22.0	93	"	"	
18.5	89	5	"	17.5	89	6	"	15.0	93	"	15.5	90	Pale yellow	17.5	87	"	"	
15.5	93	11	Considerable bleaching	15.5	94	6	"	12.5	94	"	12.5	93	" "	15.4	94	Normal	"	Yellowing

TABLE 17.—*Continued*

12.0	92	11	Slight bleaching	12.0	95	11	"	10.0	98	Color good, snap slight to fair	10.0	98	"	"	12.4	93	"	"	"
8.0	98	11	"	8.0	93	11	"	7.5	91	Color good, snap fair	7.0	91	Color and condition nearly as good as at start		10.0	98	"	Normal	"
6.0	98	11	Good condition	6.0	98	15	Good condition	2.0	97	"	2.0	97	"		7.0	93	"	"	"
2.0	98	11	"	2.0	97	15	"	1.3	95	Color good, snap excellent					6.0	95	"	"	Normal
				0.0	90	15	"												

[a] An unidentified variety shipped by express from Jensen, Florida, December 13, 1927. Arrived at Washington, D. C., Dec. 16, 1927. Stored December 17, 1927.

[b] Variety of beans used: Bountiful, from Ocala, Florida. Purchased on the Washington market, June 3, 1928. No other information regarding history of beans available. They were in good condition.

[c] Bountiful, shipped from Fort Lauderdale, Florida, January 23, 1930. Arrived at Washington, D. C., January 27 and stored January 28, 1930. Good condition.

[d] Bountiful. Shipped from Indiantown, Florida, February 22, 1930. Arrived at Washington, D. C., February 25 and stored February 26, 1930. Condition good.

[e] Bountiful. Shipped from Delray Beach, Florida, May 10, 1930. Arrived at Washington, D. C., and stored May 13, 1930.

[f] This lot of beans was also inspected after 2 days of storage, when, except for slight flabbiness at the higher temperatures, it was in good condition.

TABLE 18.—*Physiological deterioration of snap beans as related to temperature and time*[a]

Temperature	Relative humidity	Condition of beans after different periods of time in days							
		1	2	3	4	6	7	8	11
° *C.*	*Per cent*								
35.0	92	Marked yellowing	Marked yellowing	Marked rusting	Light green to rusty	Yellow and rusty	Yellow to rusty		
31.0	92	Considerable discoloration	Considerable discoloration	"	"	Light green to rusty	"		
28.5	95	"	"	Considerable discoloration	Light green	Considerable rusting	"		
25.0	91	Very slight discoloration	"	"	" "	Light green to rusty	Light yellow to rusty		
19.5	94	No discoloration	Slight discoloration	Slight discoloration, ripening	" "	Light yellow	Light yellow		
17.5	91	"	Very slight discoloration	Very slight discoloration	" "	Considerable bleaching	" "		
15.0	94	"	"	"	Slight bleaching	"	Light green to yellow		
12.0	93	"	No discoloration	Very slight bleaching	Very slight bleaching	"	"		
10.0	99	"	"	"	"	Slight bleaching	"		
7.0	93	"	"	No discoloration	No discoloration	Very slight bleaching	Very slight bleaching	Slight bleaching	Slight bleaching and rusty
2.0	97	"	"	"	"	No discoloration	"	"	"

[a] Two varieties of beans were used in these experiments. Bountiful, shipped from Fort Lauderdale, Florida, February 23, 1931, arrived at Washington February 25, 1931, and Giant Stringless Green Pod, shipped from Canal Point, Florida, February 24, 1931. Both varieties were stored at the various temperatures February 27, 1931.

readily than those of *C. lindemuthianum* and *P. butleri*. *Sclerotinia sclerotiorum, Sclerotium rolfsii,* and *Rhizoctonia solani* do not produce viable asexual spores at all, and thus are more limited in their capacity to spread. The vegetative nature of *Bacterium phaseoli* is such as to permit rapid spread. *S. sclerotiorum, R. tritici, R. nigricans,* and *P. butleri* reproduce sexually, and, in case of the last 3, oospores are produced that serve as resting bodies to bridge the fungi over unfavorable conditions. *S. sclerotiorum, S. rolfsii, R. solani,* and *B. cinerea* form sclerotial bodies that aid in perpetuating their existence from season to season. The sclerotia of all 4 organisms germinate directly into a vegetative condition. The sclerotia of *S. sclerotiorum* may germinate directly or produce apothecia whose ascospores facilitate the spread of the pathogen. In addition to being carried over from season to season in the seed, the blight pathogen is thought to be able to live over winter on bean refuse, especially in the warmer climates. The other pathogens are all able to live over in the soil. (*C. lindemuthianum* is probably short-lived in the soil.) *S. sclerotiorum, P. butleri,* and *S. rolfsii* are primarily soil organisms, and most of the primary infections probably take place by the pods coming in contact with the soil. Beans may become infected by *B. cinerea* through their contact with the soil, but infection usually takes place in transit as a result of the pods becoming contaminated with spores.

The host range of anthracnose and blight, as reported, is limited to the common bean and a few closely related species and genera. None of the susceptible cultivated crops are likely to be grown in rotation with beans. *Sclerotium rolfsii* attacks a variety of cultivated crops (beet, cabbage, corn, eggplant, potato, pepper, radish, sweetpotato, tomato, etc.) and is widely distributed throughout the South. *Sclerotinia sclerotiorum,* widely distributed in the United States, attacks a number of vegetable crops (cabbage, carrot, celery, cauliflower, eggplant, lettuce, etc.), some of which are often grown in rotation. *Rhizoctonia solani* attacks potatoes, turnips, beets, alfalfa, lettuce, cabbage, etc., and is widely distributed. *Pythium butleri* affects such crops as cucurbits, eggplant, and tomato. *Rhizopus tritici, R. nigricans,* and *Botrytis cinerea* are more or less ubiquitous and omnivorous and will attack beans in transit whenever conditions are favorable. These pathogens are less dependent on field infection and contamination than the others.

It has been assumed throughout these experiments that a high humidity in the bean container is favorable to infection and decay by the various pathogens. A relative humidity of 90 per cent and above has been provided in nearly all cases. Because of the high water content of beans, a high humidity is probably nearly always present in the shipping container. In some instances, especially when the beans are packed wet and when the

fluctuation of temperature is such as to bring about condensation, free water will be found on them in the container.

Wounding is not essential to infection of pods detached from the vines unless it be in the case of blight.

Major emphasis should be directed towards the field control of anthracnose, bacterial blight, watery soft rot, southern blight, soil rot, and cottony leak. Effective control measures are available for the first 2 diseases. All diseased beans should be sorted out and the sound beans packed when dry and kept as cool as possible. If such beans are shipped at temperatures below 12°, very little, if any, decay by Rhizopus soft rot and cottony leak would take place. There would be little, if any, loss from anthracnose at temperatures of 10° and below in less than 10 or 11 days, and none at all at temperatures below 7°. The lowest temperature at which infection by *Sclerotium rolfsii* has been obtained is 8° in 11 days. Infection by *Rhizoctonia solani* took place in 5 days at 10.5°, in 16 days at 8° and 5.5°, and in 20 days at 0.9° and 2°.

The most difficult diseases to limit and control during transit are gray-mold rot and watery soft rot. Fortunately, because of its slow rate of development, gray-mold rot is not the source of heavy losses. If beans are contaminated with *Sclerotinia sclerotiorum,* the best that can be done is to retard its development by shipping them at temperatures (0° to 5°) as low

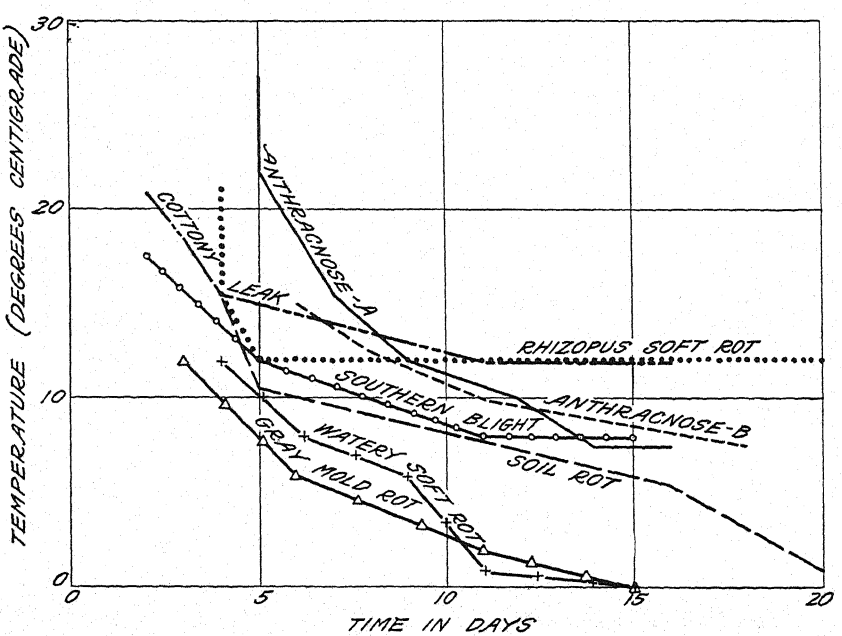


FIG. 4. Minimum time required at certain temperatures either for infection to occur or incipient infection of various diseases to become visible on beans.

as possible without incurring the danger of freezing. One bean had become infected with *S. sclerotiorum* from one point of inoculation in 4 days at 12°, 5 pods in 6 days at 8°, and infection had just started in 11 days at 2°, and in 15 days at 0°. Figure 4 illustrates the minimum time required for infection to occur at a number of temperatures in the case of all diseases except blight. In plotting these curves only cases of initial infection were used, and the graphs are based on all the results of all the experiments.

Taking into consideration the effects of the different temperatures on the quality of the beans as well as on the development of the diseases, the lower the temperature (above the freezing point of beans) employed during shipment the better the results will be.

SUMMARY

Colletotrichum lindemuthianum will infect not only wounded but also wound-free beans when the pods are sprayed with a water suspension of spores. Infection took place in beans inoculated by means of a needle prick at temperatures from 8° to 33° and in beans inoculated by spraying with a spore suspension at temperatures from 7° to 27°. Lesions have developed in visibly healthy pods sorted out from stock showing the presence of anthracnose, when stored at temperatures from 7.5° to 29°. The optimum temperature for infection ranges from 22° to 25°, the highest percentage usually occurring at 25°. The shortest time in which infection has been observed to occur at temperatures of 22°, 25°, and 27° was 5 days; at 15.5° and 17.5°, 7 days; at 12°, 9 days; at 10°, 12 days; and at 7°, 14 days. *C. lindemuthianum* spores obtained from living bean pods seem to be more virulent than those obtained from artificial cultures.

All attempts to obtain infection by spraying snap beans with a water suspension of *Bacterium phaseoli* were unsuccessful. Infections through needle pricks were obtained at temperatures from 2° to 31°. Development of blight lesions at temperatures from 1.2° to 35.0° were observed in beans free from visible signs of infection, which were sorted out from lots showing the presence of blight. The development was very slight at 35° and rather slow at temperatures of 7° and below. There was little difference in the amount of blight that developed at 10° to 24.5°.

Infection of snap beans by *Sclerotinia sclerotiorum* was obtained at temperatures from 0° to 28°. The time required for infection to occur at temperatures from 15.5° to 27.5° was not accurately determined. Five to 15 beans had been infected from one point of inoculation in 4 days at 3 temperatures. The earliest infection at 12° was observed in 4 days, at 8° in 6 days, at 5.5° to 6° in 9 days, at 1.8° to 2° in 11 days, and at 0° in 15 days. The optimum temperature for infection is approximately 19° to 24°.

The range of temperatures at which infection of snap beans has been obtained by *Pythium butleri* extends from 12° to 35.6°. Infection at 12° and 15.5° was limited to inoculated beans after 16 days' storage. The optimum temperature for infection is about 31°.

Rhizoctonia solani infected snap beans at 0.9° to 35.5°. Twenty days were required for infection to start in the inoculated beans at 0.9° and 2°, and 16 days at 5.5° and 8°. The optimum temperature for infection extends from 24° to 32°.

Infection by *Sclerotium rolfsii* was obtained on snap beans at temperatures from 8° to 35.6°, the latter being near the maximum. Infection at 8° and 12° was limited to the inoculated pod after 11 days of storage. No infection occurred at temperatures below 8°.

Infection by *Rhizopus tritici* and *R. nigricans* is not dependent on artificial inoculation and will occur to some extent in snap beans exposed to temperatures above 30°. Infection has been observed in both inoculated and noninoculated beans at temperature extending from 12° to 35.5°. Infection below 25° is usually very slight. No infection has ever been obtained at temperature below 12°.

Botrytis cinerea has infected snap beans at temperature from 0° to 35.5°. Infection occurred at 12°, 15.5°, 17.5°, 23°, 28°, and 35.5° in 4 days. Judging by the amount present at the various temperatures, infection was still in the initial stages. Infection took place at 6° and 8° in 6 days, at 2° in 11 days, and at 0° in 15 days.

Deterioration of snap beans, aside from that produced by microorganisms, is very rapid at temperatures above 20°, especially at 35.5°, and decreases with the lowering of the temperature.

In snap beans shipped from Florida to Washington, D. C., the quality remained good for 4 to 15 days at temperatures from 6° to 7° and for 6 to 15 days at temperatures below 6°.

Taking into consideration both the quality of snap beans and the diseases likely to occur in transit, shipment at temperatures below 10° is desirable and the lower the temperature (above the freezing point of beans) the better.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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SOME VIRUSES AFFECTING SPINACH, AND CERTAIN ASPECTS OF INSECT TRANSMISSION[1]

ISMÉ A. HOGGAN

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The mosaic disease of spinach (*Spinacia oleracea* L.) known as "spinach-blight" has occupied a unique position among plant virus diseases for some time, as a result of the reported transmission of the causal agent of the disease from infective parent aphids to their progeny through several generations (13). This constitutes the only evidence yet recorded that tends to indicate a hereditary association of a plant virus with the transmitting insect. Since other investigations have revealed no further evidence of a similar nature, there appears to be some question how far plant viruses may be regarded as "inheritable" by offspring of infective insect vectors. The subject is of sufficient general interest and importance in its bearing on various problems of plant-virus transmission to merit further attention, and any information tending to clarify the present situation should be of some value.

In the course of recent investigations, it has been shown (7) that the cucumber-mosaic virus is transmissible to spinach, producing symptoms on this host that closely resemble those of spinach-blight, according to the description of the latter disease by McClintock and Smith (13). On the basis of this similarity in symptoms, as well as in aphid vectors, the suggestion was made that the two diseases might be identical, and that, if this were so, further light might be thrown on the insect relationships of the spinach-blight virus. It was, therefore, decided to undertake a more critical investigation of cucumber mosaic on spinach and of its possible connection with spinach-blight, in the hope of contributing further information on the "inheritability" of this disease by the aphid vectors. Unfortunately, however, attempts to secure material of spinach-blight from the district where it was originally described have met with little success, so that it has been necessary to resort to more indirect methods in comparing the two diseases.

Since Böning (2) has recently concluded that a mosaic of spinach occurring in Germany, which he appears to regard as identical with the American spinach-blight, is probably due to the virus of beet mosaic, the present investigation has been extended to include a comparative study of this virus also in relation to spinach. Some additional work has been carried out with a third virus transmissible to this host plant, namely, that of

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

tobacco ring-spot (5, pp. 16–17). These 3 viruses have been studied primarily from the standpoint of a possible connection with spinach-blight, though certain other considerations have arisen during the investigation that have widened the scope of the work. These include problems of comparative means of transmission, of the development of local as contrasted with systemic infection, and of certain relationships between virus and aphid vector.

PREVIOUS INVESTIGATIONS

That spinach-blight, as it occurred in Virginia, was an infectious disease of the mosaic type was conclusively demonstrated by McClintock and Smith (13) in 1918. These investigators showed the disease to be readily transmissible from plant to plant by needle inoculation with diseased plant extract, as well as by several species of aphids, of which *Myzus persicae* and *Macrosiphum solanifolii* appeared to be the most important. They divided the course of the disease into 8 arbitrary stages, the characteristic symptoms of which may be summarized as follows: 1. A very slight yellowing of the younger leaves of infected plants and occasionally of one or more older leaves. 2. More pronounced yellowing of the younger leaves. 3. Malformation of the younger leaves, which become much wrinkled, narrowed, and mottled; yellowing of the older leaves and some stunting of the plant. 4. Distinct stunting of the plant, with cessation of growth; yellowing of the entire foliage; mottling of the older leaves and extreme malformation of the younger ones, which are very finely savoyed and feathery in appearance. 5. Browning and death of parts of the older leaves, usually progressing from the outer tips inwards. 6. Total disintegration of the older leaves and some browning of the central ones. 7. Browning of the younger leaves. 8. Death of the plant, which has become reduced to a very small central whorl of leaves.

The same authors presented evidence believed to indicate a direct transmission of the virus from one generation of infective aphids to another. This evidence was based in large part upon the rearing of colonies from infective parent aphids on certain host plants other than spinach, such as lettuce, pepper, eggplant, and potato, and the transfer of individuals from succeeding generations produced on these hosts back to healthy spinach. In nearly all cases, one or more of the spinach plants so treated became diseased, while needle inoculations from the foliage of the previous host of the aphids gave no infection. Similarly, transfers to healthy spinach of aphids naturally present in the field on such plants as celery, sweet potato, and wild mustard resulted in some infection with spinach-blight. In another experiment, young of virus-bearing aphids, *Macrosiphum solanifolii,* were transferred at birth to 25 healthy spinach plants, one of which became

diseased. Since the aphids causing these infections had at no time come in contact with diseased spinach, these results were interpreted as indicating a "hereditary" association of the virus with the insects.

The failure to secure infection of spinach by artificial inoculation from the leaves of the lettuce and other plants on which the infective aphid colonies were reared led the authors to conclude that it was improbable that such plants served as alternate hosts of the virus. No further conclusions were reached, however, as to the host range of the disease.

Later, L. B. Smith (18) succeeded in developing a variety of spinach that, although not strictly immune from the disease, showed a high degree of resistance to it in the field. This variety was named "Virginia Savoy."

A mosaic of spinach prevalent in Germany has been described in recent years by various writers under the name "*Mosaikkrankheit*" or "*Gelbfleckigkeit*" (3, 2, 21, 22). The characteristic symptoms of this disease are a yellowing and necrosis of the foliage, accompanied by severe stunting or death of the plant. According to Volk (21), plants infected when young rapidly become yellow all over and die, while older plants show first a curling and yellowing of the younger leaves, the discoloration then spreading over the foliage and the plant becoming much stunted. Later, the outer leaves turn brown and die, leaving a few dwarfed yellow-green leaves in the center. Eventually, the whole plant succumbs.

The opinion appears to be general among the German investigators that this disease is identical with the American spinach-blight. Thus, Schaffnit (3, p. 173), referring to the investigations of McClintock and Smith, states: " . . . *die von den Autoren* [McClintock and Smith] *beschriebene Krankheit zweifellos mit der bei uns vorkommenden identisch ist.*" Volk (21, p. 1) and Wichmann (22, p. 160) later make similar statements. This conclusion appears to be based upon a general agreement in symptoms between the 2 diseases and in transmissibility by aphids and by artificial inoculation. According to Böning (2, p. 492), however, the leaf symptoms at first consist of a number of small, usually round, yellow spots about the size of a pin's head ("*so erscheint die Spreite wie übersät mit vielen, meist runden Fleckchen von etwa Stecknadelkopfgrösse*"), and this symptom is clearly illustrated in figure 3 of his paper. This point will be referred to again later; for the present it should be noted that McClintock and Smith do not mention any discrete spotting as an early symptom of spinach-blight. Böning adds that the spots are sometimes so numerous as to coalesce, forming larger yellow areas. Of the leaf malformations described by McClintock and Smith he observed a puckering of the leaf surface and a narrowing of the lamina, particularly in the female plants in the flowering stage.

The German disease was shown to be transmissible to healthy spinach plants by aphids (*Aphis fabae* Scop. and *Macrosiphum* sp.), and less read-

ily by artificial inoculation (2). Böning was also able to transmit this mosaic to beet, and beet mosaic to spinach, by means of aphids, the symptoms developing on the respective hosts being the same for either disease. He, therefore, concluded that the two diseases were probably identical. In this connection the reader is reminded that a mosaic of both garden and sugar beet has been reported at different times by various investigators in Europe and in America (14, 4, 12, 19, 16, 1, 11).

MATERIALS AND METHODS

Upon first suspecting a relationship between cucumber mosaic and spinach-blight, attempts were made to secure material of the latter disease from Virginia and from other sections of the United States for purposes of comparison with cucumber mosaic. In spite of repeated efforts, however, no such material was obtained until the investigation was nearly completed. It, therefore, became necessary to establish certain arbitrary criteria by which the degree of resemblance of each virus under consideration to that of spinach-blight might be evaluated. The salient characteristics of the spinach-blight virus, according to the descriptions of McClintock and Smith (13), appeared to be the following: (1) Ready transmission from plant to plant both by aphids (particularly *Myzus persicae* and *Macrosiphum solanifolii*) and by artificial inoculation (needle-prick method) and (2) a characteristic sequence of symptoms on affected plants. This symptom sequence has been summarized earlier. The investigation has, therefore, consisted mainly in transmission and symptomatology studies of the respective viruses on spinach. A third criterion was based upon the resistance of Virginia Savoy spinach to spinach-blight, this variety being tested for indications of resistance to the different viruses under consideration.

Sugar-beet plants affected with mosaic were kindly furnished by P. N. Annand, Twin Falls, Idaho, and by C. W. Bennett, Riverside, California. This disease appeared to be identical with the sugar-beet mosaic described by Robbins (16). The cucumber-mosaic virus studied (*cucumber virus 1* (10)) was the same as that employed in previous transmission studies (6, 7, 8), having been obtained originally from a mosaic cucumber plant; and the tobacco ring-spot virus was derived from material received from S. A. Wingard, Virginia Agricultural Experiment Station, Blacksburg, Virginia. Seed of Virginia Savoy spinach was obtained through the courtesy of F. P. McWhorter, Virginia Truck Experiment Station, Norfolk, Virginia.

The investigation was carried out entirely in the greenhouse. Two methods of virus transmission were employed: (1) Aphid transmission by means of *Myzus persicae* and *Macrosiphum solanifolii* and (2) artificial inoculation of plant extract. The methods involved in the colonizing and handling of the aphids were similar to those employed in previous trans-

mission studies and have been described in detail elsewhere (6, 8). Virus-free colonies of *Myzus persicae* were maintained on cabbage and those of *Macrosiphum solanifolii* on potato. In aphid-transmission trials, it was customary to transfer to the control plants a number of nonviruliferous aphids direct from the stock colonies or from other healthy plants.

Two methods of artificial inoculation were employed. In the "cotton-needle" method, a small quantity of absorbent cotton was wrapped around the tip of an ordinary dissecting needle, and this was dipped into the infectious plant extract and used to scratch and prick the leaf lamina and midrib. The second method was essentially the rubbing method of Holmes (9), the leaf surface being wiped with a piece of cheesecloth moistened with virus extract. Samuel (17) has recently pointed out that this is a very effective means of securing infection with several viruses. With smooth-leaved plants, such as sugar beet and spinach, however, a heavy rubbing that bruised without actually tearing the leaf, amounting almost to a "leaf mutilation" inoculation, proved more effective than a light wiping and was practiced throughout for smooth-leaved plants. For convenience, both variations of this method will be referred to as the "rubbing" method, following Holmes's terminology. Plants serving as controls in artificial inoculation series usually received no treatment.

The following host plants were used during the investigation: Tobacco, *Nicotiana tabacum* L., Havana Seed type; spinach, varieties Bloomsdale and Virginia Savoy; sugar beet, *Beta vulgaris* L., unknown variety; and cucumber, *Cucumis sativus* L., variety Select White Spine. Vigorous seedlings were used in all trials. The spinach and sugar beet were grown in a cool greenhouse (65–70° F.), the tobacco and cucumber in a warmer house (80–85° F.). Most of the work involving spinach was conducted in the early spring and fall months, when light and temperature conditions favored good growth. This plant made very poor growth during the winter and tended to rapid flower-stalk formation during the longer and warmer days of late spring and summer.

Each transmission trial was repeated several times, a total of 20 plants usually being tested for each type of virus transfer. It was believed that the results obtained in this way, from several repetitions of each experiment, were more significant than when fewer trials were made, each involving larger numbers of plants.

EXPERIMENTAL RESULTS

The suggestion advanced by Böning (2) of a probable relationship between spinach mosaic and beet mosaic immediately raised the question of the specificity of the virus or viruses responsible for the latter disease. So far as could be judged from the description of symptoms of both beet mo-

saic and sugar-beet mosaic published by different European and American investigators (14, 16, 1, 11), it appeared likely that the same virus was involved in most, if not all, cases. Almost no information was available, however, on the host range of the disease or on any other diagnostic characters of the virus beyond the fact that it was transmitted by aphids (16, 1). Since cucumber mosaic was found to be transmissible to sugar beet, it seemed conceivable that the cucumber-mosaic virus might be identical with that causing sugar-beet mosaic. While this subsequently proved not to be the case, a comparison of the two viruses on sugar beet appeared to be a necessary preliminary to the main investigation.

Comparison of Viruses on Sugar Beet and Tobacco. The cucumber-mosaic virus was found to be readily transmissible from tobacco to sugar

TABLE 1.—*Transmission of the cucumber mosaic virus to sugar beet and spinach*[a]

Mosaic host (source of virus)	Host	Mode of transmission							
		Myzus persicae		*Macrosiphum solanifolii*		Needle inoculation		Rubbing inoculation	
		Infested	Control	Infested	Control	Inoculated	Control	Inoculated	Control
Tobacco	Sugar beet	30/30	30/0	20/20	20/0	20/0	20/0	20/0(19)	20/0
	Spinach var. Bloomsdale	30/28	30/0	20/20	20/0	20/19	20/0	20/20	20/0
	Spinach var. Virginia Savoy	40/17	40/0	40/19	40/0	20/0	20/0	20/0	20/0
Sugar beet	Tobacco	20/19	20/0	20/17	20/0	20/9	20/0	20/20	20/0
Spinach var. Bloomsdale	Spinach var. Bloomsdale	20/20	20/0	25/25	25/0	30/28	30/0	20/20	20/0
	Tobacco	20/20	20/0	20/17	20/0	20/9	20/0	20/20	20/0
Spinach var. Virginia Savoy	Spinach var. Virginia Savoy	20/15	20/0	20/10	20/0	20/0	20/0	20/0	20/0
	Tobacco	20/14	20/0	20/8	20/0	20/7	20/0	20/20	20/0

[a] "Infested" denotes plants to which aphids were transferred from a mosaic host; "inoculated" denotes plants artificially inoculated with virus extract; and "control" denotes plants serving as controls to those either infested or inoculated. In all fractions, the numerator represents the total number of plants infested with aphids, artificially inoculated or treated as controls as the case may be; the denominator, the number of plants becoming infected. Figures in parentheses denote plants developing local infection only.

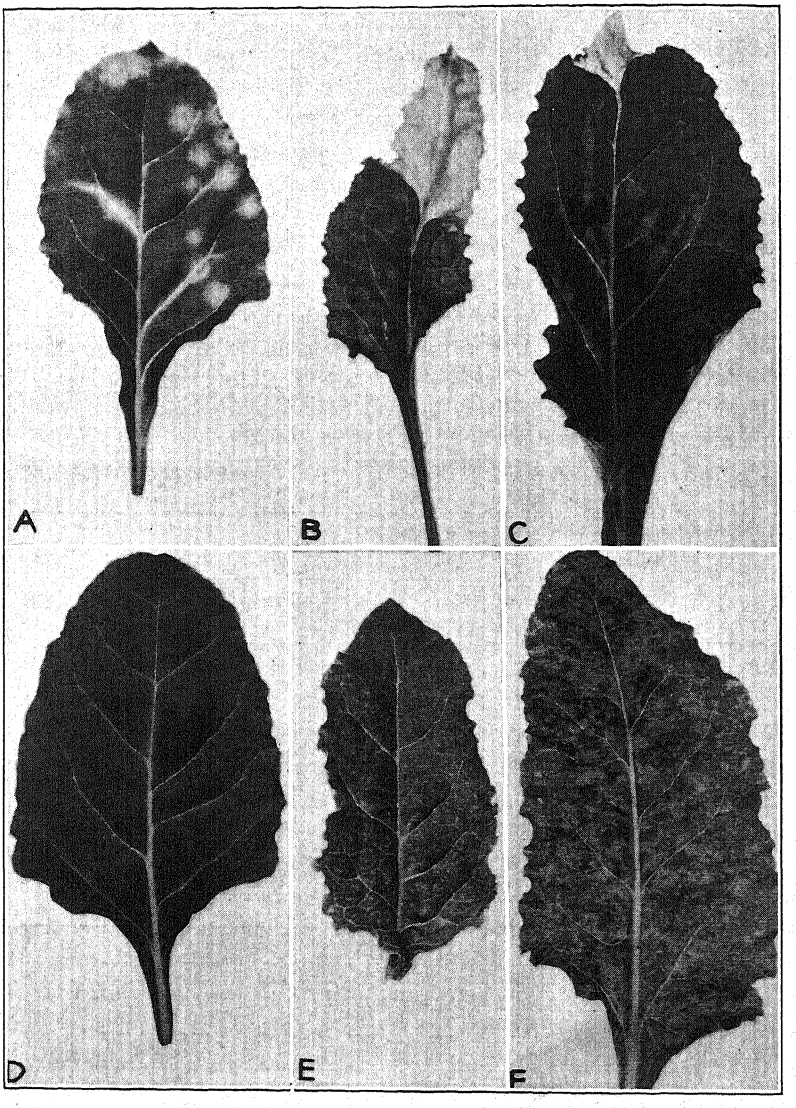


FIG. 1. Leaf symptoms produced by viruses on sugar beet. A. Local lesions of cucumber mosaic transmitted by *Myzus persicae*. B and C. Cucumber mosaic. Chlorosis due to systemic infection. D. Leaf of normal plant. E. Sugar-beet mosaic, early symptoms of systemic infection. F. Sugar-beet mosaic, later symptoms.

beet, and *vice versa*, by means of the aphids *Myzus persicae* and *Macrosiphum solanifolii* (Table 1), species that have been shown elsewhere to be efficient vectors of this virus to other hosts (6, 8). When, following the usual procedure in aphid-transmission trials, viruliferous aphids were al-

lowed to feed freely on the foliage of young sugar-beet plants systemic infection of the plants was readily obtained. The first sign of infection, however, was the appearance of conspicuous, chlorotic spots on the infested leaves, 5 or more days after the transfer of viruliferous aphids to the plant (Fig. 1, A). These spots were evidently of the nature of "local lesions" and appeared to develop at points of introduction of the virus into the tissues by the aphids. They were never observed on leaves on which the aphids were known not to have fed nor on leaves on which only nonviruliferous aphids had fed. When first observed, the lesions were pale yellow green, usually circular, though sometimes irregular in form, 2 mm. or more in diameter, and generally with a very diffuse margin. They grew rapidly larger, becoming brighter yellow and frequently coalescing to form irregular, rather indefinite, chlorotic areas. Sometimes a minute necrotic dot was visible at the center of the spot.

Symptoms of systemic infection became apparent one or more days after the appearance of the local lesions. These consisted essentially in a chlorosis of the whole or part of the newly developing leaves, successive leaves generally showing progressively smaller chlorotic areas, situated at the apical end of the leaf (Fig. 1, B, C). Chlorosis of this type is characteristic also of the earlier stages of infection of tobacco with the same virus. Occasionally, dark green, savoyed areas were present on such leaves. Leaves developing later showed a coarse mottling of relatively large light and dark areas, accompanied by considerable stunting of the plant. Plants thus infected continued to put forth mottled leaves throughout the season's growth. The virus was readily recovered from such plants onto tobacco both by means of aphids and by the rubbing method of artificial inoculation, and less readily by needle inoculation of extracted juice (Table 1).

Systemic infection of sugar beet with the cucumber-mosaic virus was not secured, however, by either method of artificial inoculation (Table 1). Local symptoms, more or less similar to the local lesions resulting from infection by aphids, were usually observed on leaves that had been inoculated by the rubbing method,[2] and the virus was later recovered from these leaves by means of inoculation of extracted juice to tobacco. Since the cucumber-mosaic virus is known to lose its infectivity within a few days outside the living host, it is evident from these results that the virus had been able to establish itself in the inoculated leaves. No virus was recovered at any time, however, from *uninoculated* leaves of the same plants, even after 3 or more weeks had elapsed; in other words, there was no systemic infection of plants following artificial inoculation.

[2] Samuel (17, p. 502) has also reported local infection of sugar beet with the cucumber-mosaic virus, following a similar method of inoculation.

A similar condition obtained when viruliferous aphids, instead of being allowed to wander freely over the plant, were confined to a single leaf within a glass tube closed at both ends. Local lesions, resembling those previously described, developed under these conditions on the infested leaf, enlarging and coalescing until the whole leaf became yellow and eventually died. The virus was readily recovered from such leaves by the rubbing method of inoculation. However, no systemic infection of the plants followed, all uninfested leaves remaining normal in appearance and free from virus, as tested by subsequent inoculations to tobacco.

Explanation of this peculiar situation is at best only tentative. For some reason, under the conditions of the experiment, the cucumber-mosaic virus appears unable to pass from a single infected leaf of this host to other parts of the plant. The systemic infection obtained when the aphids were not restricted to any particular organ may possibly have resulted from a direct introduction of virus at or near the growing point of the shoot, the virus perhaps multiplying at this point and forming a source of infection for all subsequent growth. In this connection it was observed that the bud was a favorite feeding point of the aphids.

Comparison of the symptoms of cucumber mosaic on sugar beet with those of sugar-beet mosaic soon showed that the two diseases were not the same, although both proved transmissible by the same species of aphids. Sugar-beet mosaic has been reported by Robbins (16) to be transmissible by *Myzus persicae,* and in the present investigation ready transmission of the virus was obtained on sugar beet by means of this aphid, and also with *Macrosiphum solanifolii.* With either species, 100 per cent infection was secured in all trials, all control plants remained healthy (Table 2). The virus was found transmissible also by artificial inoculation, particularly when a severe rubbing method was used. Thus, of 20 plants tested by either method, 18 were infected by rubbing and 10 infected by needle inoculation, systemic infection developing in all cases, while all control plants remained healthy (Table 2). In this respect the virus differed from that of cucumber mosaic, with which systemic infection of sugar beet was not secured through artificial inoculation.

It may be noted that several previous investigators (16, 1, 11) have reported failure to obtain transmission of beet mosaic or of sugar-beet mosaic by methods of artificial inoculation. Assuming the same virus to be involved in all cases, this apparent inconsistency in results may no doubt be ascribed to differences in the type of technique employed. Verplancke (20), on the other hand, claims to have transmitted beet mosaic by rubbing the leaves of healthy plants with mosaic-plant extract.

Systemic infection with sugar-beet mosaic resulted also when viruliferous aphids were confined to single leaves of healthy sugar-beet plants. This

TABLE 2.—*Transmission of the sugar-beet mosaic virus to sugar beet and spinach*[a]

Mosaic host (source of virus)	Host	Mode of transmission							
		Myzus persicae		*Macrosiphum solanifolii*		Needle inoculation		Rubbing inoculation	
		Infested	Control	Infested	Control	Inoculated	Control	Inoculated	Control
Sugar beet	Sugar beet	20/20	20/0	20/20	20/0	20/10	20/0	20/18	20/0
	Spinach var. Bloomsdale	20/20	20/0	20/20	20/0	20/15	20/0	20/20	20/0
	Spinach var. Virginia Savoy	20/20	20/0	20/19	20/0	20/13	20/0	20/18	20/0
	Tobacco	20/0(20)	20/0	10/0(10)	10/0	20/0	20/0	20/0	20/0
Spinach var. Bloomsdale	Spinach var. Bloomsdale	20/20	20/0	20/17	20/0	20/12	20/0	20/16	20/0
	Sugar beet	20/20	20/0	20/20	20/0	20/7	20/0	20/17	20/0
Spinach var. Virginia Savoy	Spinach var. Virginia Savoy	20/20	20/0	20/19	20/0	20/12	20/0	20/17	20/0
	Sugar beet	20/20	20/0	20/19	20/0	20/9	20/0	20/20	20/0

[a] See footnote to table 1.

contrasts again with the behavior of the cucumber-mosaic virus, which caused only local infection under similar conditions, and would seem to indicate some distinct difference in the host-tissue relationships of the respective viruses.

Since the symptoms of sugar-beet mosaic have been described in detail by Robbins (16), only the more important distinguishing features will be considered at the present time. On young sugar-beet plants, the first symptoms observed were usually those of systemic infection; though under certain conditions, in aphid transmission trials, these were preceded by the appearance of faint chlorotic spots on the infested leaves, presumably arising at points where the aphids had fed. These lesions were about 1 mm. in diameter and remained much smaller and less conspicuous than those associated with local infection of the same host with cucumber mosaic.

Systemic infection became apparent in the form of minute yellow or light green flecks on the young leaves 5 or more days after infection (Fig. 1, E). These flecks were less than 1 mm. in diameter and were situated for

the most part over the veins. Sometimes they were so numerous as to give temporarily the effect of a marked clearing of the veins. They increased somewhat in size, the mosaic pattern soon becoming resolved into a sharply defined, fine, speckly mottling, quite different from the chlorosis or coarse, indefinite mottling characteristic of cucumber mosaic on this host. Leaves of later development usually showed a similar type of mottling (Fig. 1, F), though sometimes larger light and dark areas were formed. These differences in the symptoms produced by the 2 viruses should be clear from a comparison of the illustrations to which reference has been made.

A further comparison of the 2 viruses was made on tobacco, a host already known to be susceptible to cucumber mosaic. No infection of tobacco with the sugar-beet-mosaic virus was obtained, however, by either method of artificial inoculation. On the other hand, when attempts were made to transfer this virus by means of aphids, certain symptoms developed on the infested leaves of the tobacco plants, apparently at points where the aphids had fed, although systemic infection did not follow. These local symptoms consisted of small, round, light brownish yellow spots on the leaf lamina, 1 to 2 mm. in diameter, with very diffuse margins and often with a minute necrotic point in the center (Fig. 2, A). That these represented centers of multiplication of the sugar-beet-mosaic virus within the leaf was indicated by the fact that the virus could readily be recovered from such leaves by rubbing inoculation of extracted juice to healthy sugar beet. Moreover, tobacco plants infested with nonviruliferous aphids from healthy sugar beet showed no spotting. The lesions were first visible 3 or more days after infestation of the plants with infective aphids and enlarged quite rapidly to about 1 cm. in diameter, later becoming gradually less distinct. Sometimes they coalesced to form a diffuse yellowish pattern on the leaf. The spots showed a marked tendency to necrotic ring formation, often developing 2 or more fine, concentric rings (Fig. 3). Similar, though less numerous, lesions were produced on tobacco by viruliferous individuals of *Macrosiphum solanifolii*. On the other hand, all uninfested leaves of the same tobacco plants remained normal in appearance and free from all symptoms of disease, nor could any virus be recovered from them, even several weeks after the appearance of the lesions on infested leaves. It was, therefore, concluded that systemic infection had not taken place. The symptoms produced by this virus on tobacco are entirely distinct from those of cucumber mosaic, with which systemic infection is readily secured both by aphids and by artificial inoculation, resulting in a very different type of chlorosis and mottling of the leaves.

From this preliminary comparison of cucumber mosaic and sugar-beet mosaic it is concluded that the 2 diseases differ considerably from one an-

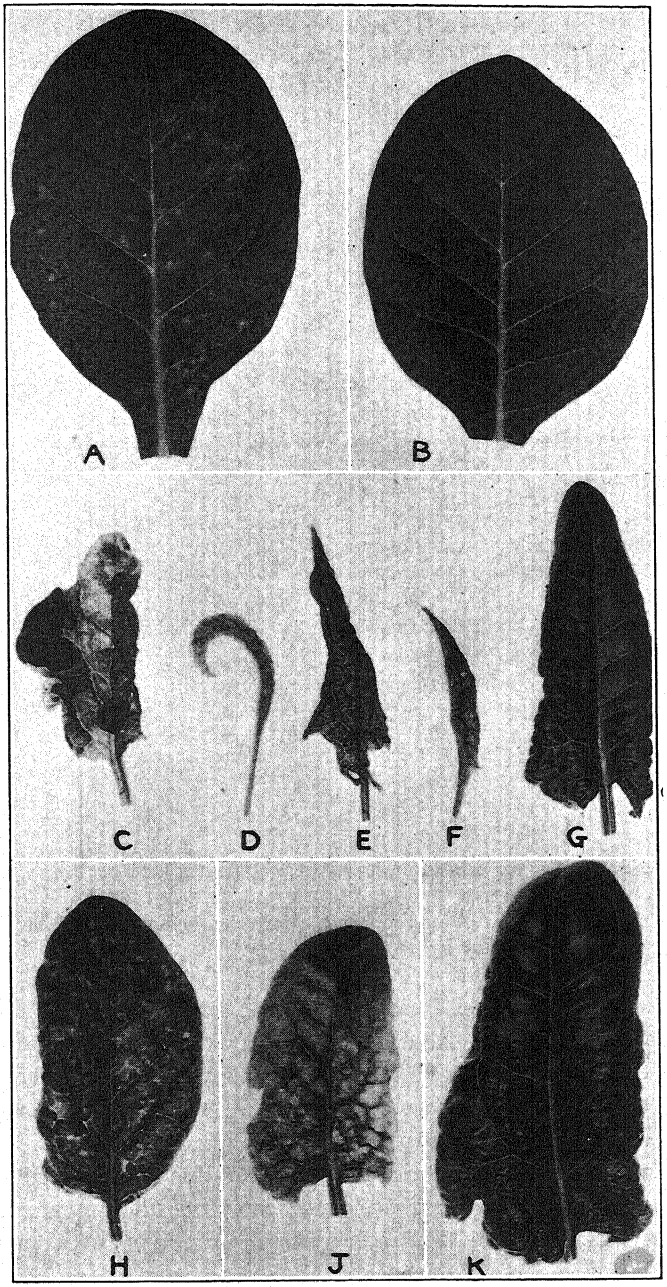


FIG. 2. Leaf symptoms produced by viruses on tobacco and spinach (variety Bloomsdale). A. Local lesions of sugar-beet mosaic on tobacco, following transmission by *Myzus persicae*. B. Leaf of normal tobacco plant. C–F. Cucumber mosaic on spinach, showing mottling and malformation of young leaves. G. Corresponding leaf of normal spinach plant. H. Early symptoms of sugar-beet mosaic on spinach. J. Tobacco ring-spot on spinach. K. Corresponding leaf of normal spinach plant.

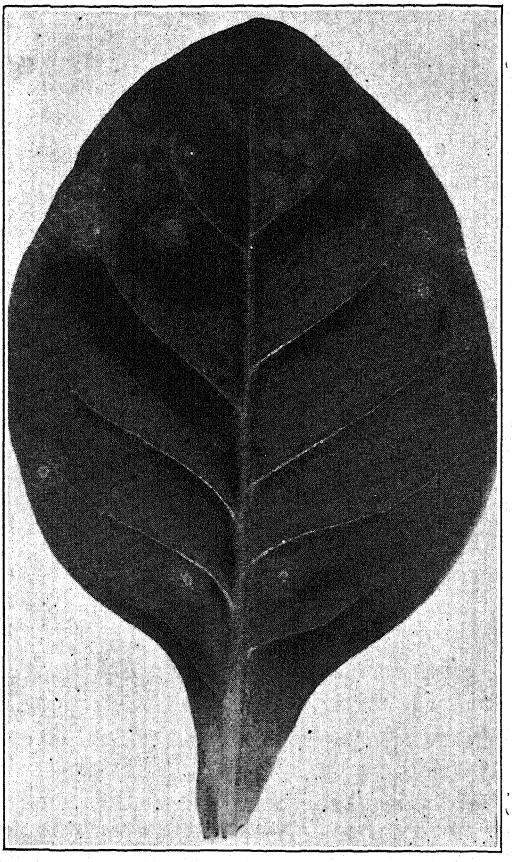


FIG. 3. Local lesions of sugar-beet mosaic on tobacco resulting from infection by *Myzus persicae*, showing development of necrotic rings.

other in their behavior and symptoms, both on sugar beet and on tobacco, and it seems clear that 2 quite distinct viruses are concerned.

In this connection it may be well to summarize briefly the characteristic symptoms on these 2 host plants of the third virus under consideration, namely, that of tobacco ring-spot. On tobacco, the ring-spot virus typically produces an intricate necrotic ring and line pattern, consisting of concentric rings and parallel zigzag lines, sometimes accompanied by mottling of the leaf and associated with systemic infection of the plant (23). On sugar beet, Wingard (23) reports the development of faint zigzag lines on the leaves, of lighter color than the normal tissue, but not necrotic. Similar symptoms, representing systemic infection, have been observed on this host by the writer, though in some cases the lines became necrotic.

Comparison of Viruses on Spinach. Following the preliminary comparison of viruses on sugar beet and tobacco, it was now possible to proceed to a study of their symptoms and behavior on spinach, with the object of comparing each virus with that causing spinach-blight. As previously indicated, the chief known characteristics of the spinach-blight virus appeared to be its ready transmission both by aphids and by artificial inoculation, its symptom sequence on spinach, and the resistance to infection manifested by the Virginia Savoy variety.

The results of transmission trials with the cucumber-mosaic virus on spinach are summarized in table 1. Throughout this investigation the variety Bloomsdale has been used as representing a variety of spinach susceptible to spinach-blight. When this variety was tested, the cucumber-mosaic virus was found to be very readily transmissible from tobacco to spinach and from spinach to spinach by means of either *Myzus persicae* or *Macrosiphum solanifolii* and also by both needle and rubbing methods of inoculation. With each method of transmission, 100 per cent infection was almost always obtained. The virus was also readily recovered from Bloomsdale spinach onto tobacco by means of aphids and inoculation by rubbing and less readily by needle inoculation. In all trials, the control plants remained free from infection.

The sequence of symptoms of cucumber mosaic on Bloomsdale spinach, occurring on young plants under the greenhouse conditions prevalent during the investigation, was found to correspond remarkably closely to that described for spinach-blight by McClintock and Smith (13). The symptoms developed gradually, the incubation period varying greatly according to the time of year and the prevailing environmental conditions. The minimum time observed between inoculation and the first appearance of recognizable symptoms was 7 days for infection by aphids and 12 days for artificial inoculation. This corresponds with the minimum period of 7 days mentioned by McClintock and Smith for spinach-blight. As with their disease, however, incubation periods of 2 weeks or more were quite common under certain conditions of light and temperature.

The first recognizable symptom produced by the cucumber-mosaic virus was a very faint, general chlorosis, sometimes involving the entire foliage, sometimes, especially with older plants, apparent only on the younger leaves. As growth continued, this chlorosis became more intense and spread to the outer leaves also, if these were not originally affected, until at a later stage of the disease the entire plant became bright yellow. At the same time the newly developing leaves began to show evidence of malformation in varying degrees. In milder cases, this malformation consisted in a reduction in width of the lamina, frequently accompanied by considerable wrinkling and an inward rolling of the leaf margin (Fig. 2, E, F).

In more severe cases, some leaves became reduced to a fine ribbon (Fig. 2, D); others were much puckered, twisted, or distorted; while some developed a mottling with marked savoying of the darker areas (Fig. 2, C). Similar types of malformation have been described by McClintock and Smith (13) in association with spinach-blight. As the yellowing and malformation progressed, the plant became much stunted and growth was increasingly retarded. A plant at this stage, showing discoloration of the entire foliage and slight malformation of the inner leaves, with marked inward rolling of the margins, is illustrated in figure 4, A. Later, a progres-

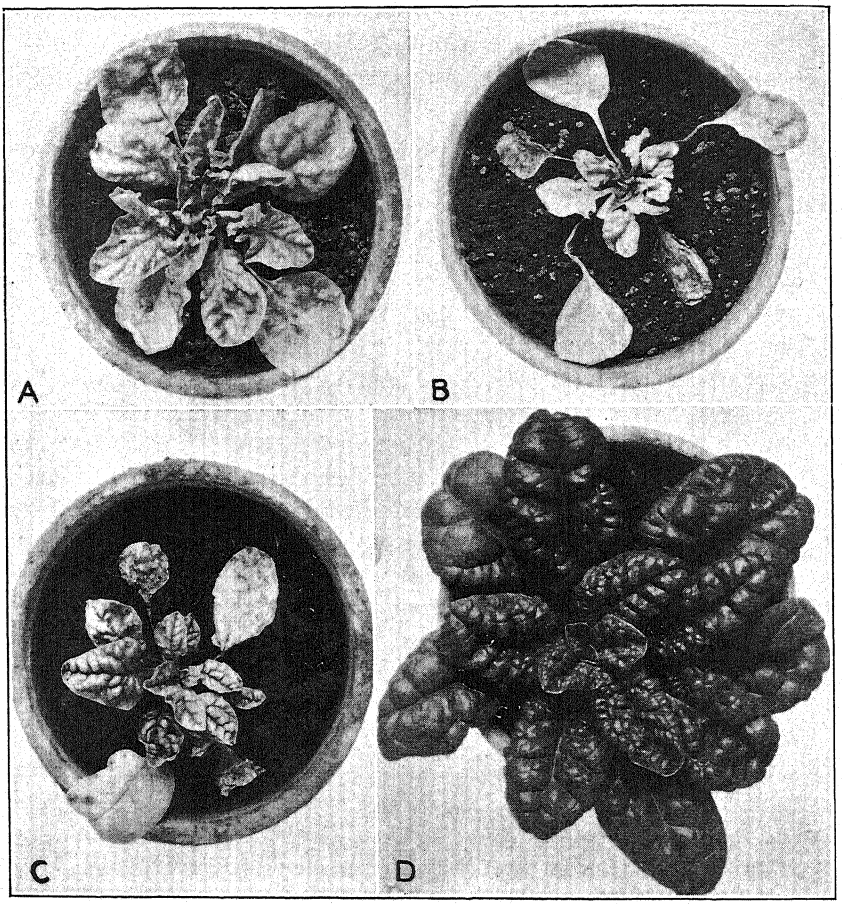


FIG. 4. Symptoms produced by viruses on spinach (variety Bloomsdale). A. Cucumber mosaic, moderately advanced stage of disease. B. Cucumber mosaic, later stage, showing necrosis of outer leaves. C. Tobacco ring-spot, severe infection. D. Normal plant of approximately the same age as plants A and C.

sive necrosis of the leaves developed, involving first the outermost whorl and slowly working inwards. As the affected leaves turned brown and shriveled and growth declined, the plant became gradually reduced in size (Fig. 4, B), until only a small central cluster of yellow, malformed leaves remained. Finally the whole plant died. These symptoms, both in character and sequence, are essentially the same as those described by McClintock and Smith for spinach-blight (13).

In tests of the susceptibility of the Virginia Savoy variety to cucumber mosaic, variable results were obtained, depending apparently upon the season and the resulting environmental conditions. At times it was found possible to secure a high percentage of infection of this variety by means of aphids, while at other times, following an identical procedure, no infection of the plants took place. Thus, of a total of 80 Virginia Savoy plants tested at various times with aphids from cucumber-mosaic tobacco, 17 out of 40 became infected by means of *Myzus persicae* and 19 out of 40 by *Macrosiphum solanifolii* (Table 1). On the other hand, parallel trials performed simultaneously with Bloomsdale spinach invariably yielded extremely high percentages of infection with aphids under all conditions. Similarly, transmission of the virus by aphids could be secured at times from Virginia Savoy to Virginia Savoy, though less consistently than with Bloomsdale. Moreover, in all earlier trials, artificial inoculation of Virginia Savoy, by either needle or rubbing method, failed to yield infection of any plants, whether diseased tobacco or Virginia Savoy was used as the source of inoculum; although in parallel trials, conducted at the same time, Bloomsdale was readily infected by these means. Thus, of a total of 40 Virginia Savoy plants tested for either method of artificial inoculation all remained healthy (Table 1). At the close of the investigation, however, in a repetition of these trials, some infection of very young Virginia Savoy plants was obtained through the rubbing method of inoculation, tobacco being used as the source of inoculum. These results are not included in table 1. In further trials the virus was readily recovered from diseased Virginia Savoy by means of aphids and rubbing inoculation and less readily by the needle method, all control plants remaining free from infection throughout. From these results it is, therefore, concluded that, although Virginia Savoy may be susceptible to cucumber mosaic under certain greenhouse conditions, this variety shows much greater resistance to the disease than does the variety Bloomsdale.

The onset of symptoms of cucumber mosaic was much the same on Virginia Savoy as on Bloomsdale, though in the former variety the yellowing and necrosis usually progressed much more quickly, necrosis of the bud soon setting in, resulting in rapid death of the plant (Fig. 5, C). Consequently, as a rule, very little growth was made after the first appearance

of symptoms, and the stage of malformation of newly developing leaves did not always occur. Older plants, however, which usually died less rapidly, developed very conspicuous malformation of the young leaves, these being reduced to small, yellow, almost ribbon-like structures.

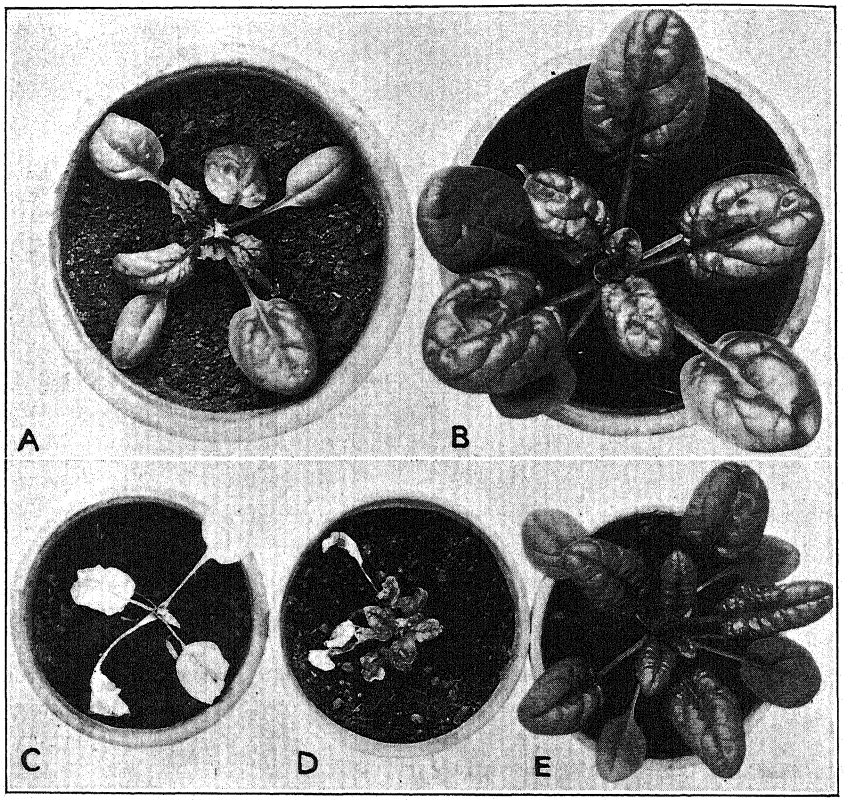


FIG. 5. Symptoms produced by viruses on spinach (variety Virginia Savoy). A. Sugar-beet mosaic, early stage of disease. B. Normal plant, control to A. C. Cucumber mosaic transmitted by *Myzus persicae*, showing rapid death of plant. D. Sugar-beet mosaic, late stage of disease. E. Normal plant, control to C and D.

The actual factors governing resistance or susceptibility of Virginia Savoy to infection with cucumber mosaic have not been determined, although there was some indication that both air temperature and light intensity might influence the amount of infection secured and possibly also the size of the plants at the time of inoculation. It should be pointed out that Virginia Savoy is not reported as immune from spinach-blight, though it exhibits a relatively high degree of resistance to the disease in the field as compared with other commercial varieties (18). In this respect, there-

fore, there appears to be considerable resemblance between the virus causing spinach-blight and that of cucumber mosaic. It has already been shown that the 2 diseases are strikingly similar in symptom sequence and also in ease of transmission on suspectible varieties, both by artificial inoculation and by aphids. While similarity in symptoms alone is not to be regarded as conclusive evidence of the identity of 2 viruses, this factor, when coupled with close agreement in other respects as well, should afford a more reliable indication of relationship. On the basis of these considerations, therefore, there appears to be very strong evidence in favor of the conclusion that the 2 diseases are very closely related, if not identical. On the other hand, sugar-beet mosaic on spinach, while resembling spinach-blight in some respects, shows also some distinct differences, both in its symptom complex and in the lack of any indications of resistance to the disease in the Virginia Savoy variety.

The results of transmission trials with sugar-beet mosaic on spinach are included in table 2. As with cucumber mosaic, the virus was found to be readily transmissible to Bloomsdale spinach and from spinach to spinach by means of both species of aphids and inoculation by rubbing. It appeared, however, to be somewhat less readily transmissible from plant to plant by needle inoculation than was the cucumber-mosaic virus, 60 to 75 per cent infection with sugar-beet mosaic being obtained by this method of inoculation as compared with 94 to 95 per cent infection with cucumber mosaic. Moreover, the sugar-beet-mosaic virus proved to be as readily transmissible to Virginia Savoy, from both infected sugar beet and Virginia Savoy, as it was to Bloomsdale, by means of aphids and by artificial inoculation, although in parallel trials with the cucumber-mosaic virus little or no infection of Virginia Savoy might be secured. Thus, there was no indication of any resistance in Virginia Savoy to sugar-beet mosaic, a point that suggests a distinct difference between this disease and spinach-blight. In further trials, the virus was readily recovered onto sugar beet from both varieties of spinach by means of aphids and by rubbing inoculation and less readily by needle inoculation. In all trials with this virus, all control plants remained free from infection.

The symptoms of sugar-beet mosaic on spinach appeared to be identical on both Bloomsdale and Virginia Savoy, and the following description of the disease is applicable to either variety. As with cucumber mosaic, the incubation period varied greatly according to the time of year and the prevailing environmental conditions. The minimum period observed was 6 days for infection by aphids and 7 days for artificial inoculation, although periods of 2 to 3 weeks or more were quite common under less favorable conditions.

The onset of symptoms of sugar-beet mosaic on spinach was quite sudden. The first sign of infection was usually a marked downward arching

of the young leaves, accompanied or soon followed by the appearance of numerous bright golden yellow flecks on the young leaves (Figs. 2, H, and 5, A). These flecks were round or irregular in form, less than 1 mm. in diameter, often with a minute darker point in the center. Frequently they became so numerous as to coalesce, forming large yellow areas. There was no faint uniform chlorosis of the foliage at this stage, as there was with cucumber mosaic, but always a distinct spotting, of a type not mentioned by McClintock and Smith as an early symptom of spinach-blight, although very similar to the mottling described and illustrated by Böning (2) as an early symptom of the spinach mosaic occurring in Germany. Indeed, the symptoms illustrated in figure 3 of Böning's paper (2) appear almost identical with those of sugar-beet mosaic on spinach, as shown in figure 2, H, of the present paper. Leaves of later development showed similar symptoms and were considerably dwarfed and puckered, although undergoing no marked malformation such as has been described for spinach-blight (13). In this connection it may be noted that Böning (2), while discussing the symptoms of the German spinach mosaic, describes, as types of malformation observed, only a puckering of the leaf and a narrowing of the lamina, particularly in older plants at the flowering stage.

As the disease progressed, the yellow coloration gradually spread over the foliage, the plant became much stunted, and growth soon ceased. Necrosis of the outer leaves then developed, as with cucumber mosaic, the leaves usually dying back from the tip. This necrosis spread slowly inward until the plant became reduced to a central whorl of small, mottled, and puckered leaves (Fig. 5, D) and finally died. Plants of the Bloomsdale variety, especially when very young, usually succumbed more rapidly to sugar-beet mosaic than to cucumber mosaic, except at higher temperatures, when the symptoms of sugar-beet mosaic were less severe and the plants tended to survive. At certain stages, however, there might be a decided resemblance between the 2 diseases, especially on older plants, and, if the malformation associated with cucumber mosaic was not marked, it sometimes required considerable experience to distinguish between the 2 on the basis of symptoms alone.

From the foregoing description it will be seen that sugar-beet mosaic on spinach, while closely resembling spinach-blight in certain respects, differs from it in the occurrence of a fine, discrete spotting as an early symptom of the disease and in the absence of any marked malformation of the leaves of seedling plants such as is associated with "spinach-blight. When one considers also the less ready transmissibility of the virus on spinach by needle inoculation and the fact that Virginia Savoy shows no evidence of resistance to sugar-beet mosaic, it becomes apparent that this disease bears less resemblance to the originally described spinach-blight than does

cucumber mosaic. On the other hand, sugar-beet mosaic on spinach resembles so closely the mosaic of spinach described by various German authorities (3, 2, 21, 22) as to suggest a probable identity of these 2 diseases, thus supporting the contention of Böning (2) that the spinach mosaic prevalent in Germany is identical with beet mosaic.

When the tobacco ring-spot virus was found to be transmissible to spinach, it was thought that some comparative tests of the symptoms and behavior of this virus also on spinach might be of interest, since the disease is known to be prevalent on other hosts in certain districts. Only a few trials were conducted, however, as it was soon shown to differ in several respects from spinach-blight.

Tobacco ring-spot is known to be a disease of wide host range, readily transmissible by certain methods of artificial inoculation (23). Up to the present, however, no insect vectors of the virus have been determined. In the family Chenopodiaceae, garden and sugar beet are known to be susceptible to the disease (15, 23), though, so far as the writer is aware, spinach, *Spinacia oleracea* L., has not previously been recorded as a host of the virus.

The tobacco ring-spot virus proved very readily transmissible from tobacco to spinach and from spinach to spinach (var. Bloomsdale) by both rubbing and needle methods of artificial inoculation (Table 3). In limited tests, Virginia Savoy appeared to be equally susceptible to infection by these means. The virus was also readily recovered from Bloomsdale spinach to tobacco through inoculation by rubbing, although infection was

TABLE 3.—*Transmission of the tobacco ring-spot virus to spinach*[a]

Mosaic host (source of virus)	Host	Mode of transmission							
		Myzus persicae		*Macrosiphum solanifolii*		Needle inoculation		Rubbing inoculation	
		Infested	Control	Infested	Control	Inoculated	Control	Inoculated	Control
Tobacco	Spinach var. Bloomsdale					15/15	15/0	15/15	15/0
	Spinach var. Virginia Savoy					10/10	10/0	10/10	10/0
Spinach var. Bloomsdale	Spinach var. Bloomsdale	20/0	20/0	20/0	20/0	10/10	10/0	10/10	10/0
	Tobacco	10/0	10/0	10/0	10/0	10/0	10/0	10/10	10/0

[a] See footnote to table 1.

not obtained in this transfer by means of needle inoculation. No infection resulted in any trials following attempts to transmit the virus by aphids (*Myzus persicae* and *Macrosiphum solanifolii*); in this respect the virus differed markedly from that of spinach-blight.

The minimum incubation period observed on spinach was 9 days. The first symptoms were those of systemic infection, large chlorotic areas appearing on the young leaves, sometimes becoming bright yellow (Fig. 2, J). These areas showed a marked tendency to necrosis, the entire affected region usually drying out very rapidly. No malformation of the leaves was observed. The plants showed a tendency to recover, especially in warm weather, subsequently developing leaves often being almost free from symptoms. Death of the plant did not occur under the conditions prevailing during the trials, although occasionally the entire foliage became yellow and the plant much stunted (Fig. 4, C). This disease, therefore, did not appear to bear a very close resemblance to spinach-blight. It is significant, however, that all 3 viruses studied in the present connection characteristically produce conspicuous yellowing and necrosis of the leaves of this host and that 2 at least may cause death of the plant.

Isolation of Cucumber Mosaic from Naturally Infected Spinach. Toward the close of the investigation here reported, a number of spinach plants of a Savoy type, said to be naturally infected with spinach-blight, were kindly sent by E. E. Clayton from the Long Island Vegetable Research Farm of the New York Agricultural Experiment Station, Riverhead, New York. Ten of these plants were selected at random and attempts were made to isolate from them any virus or viruses responsible for the diseased condition. This was accomplished by means of a transfer of aphids from each plant to healthy Bloomsdale spinach and, later, as soon as symptoms of disease appeared, from these spinach plants to tobacco and to sugar beet. Artificial inoculations were made also from the original spinach plants to tobacco and sugar beet. By these means a virus was recovered from all 10 plants whose symptoms on the various hosts closely resembled those of cucumber mosaic. A direct comparison was, therefore, made of the symptoms produced by this virus on tobacco and on cucumber with those of known cucumber-mosaic virus (*cucumber virus 1* (10)) by means of parallel inoculations to young cucumber and tobacco plants. On each host, the symptoms produced by either virus were almost identical. A comparison was made also of the thermal death point and longevity *in vitro* of either virus in tobacco-plant extract. In these determinations the thermal death point for each virus was found to lie between 65 and 70° C. and the longevity *in vitro* between 1 and 2 days. On the basis of these results, the virus isolated from the spinach plants was, therefore, concluded to be *cucumber virus 1*. No sugar-beet-mosaic virus was recovered from any of

the plants, as was indicated by the absence of any symptoms of this virus on any of the hosts inoculated; nor was the presence of any other virus detected. Unfortunately, no further material of spinach-blight has been secured at any time, so that too much significance should not be attached to these results. They show, however, that cucumber mosaic may affect spinach in the field, producing symptoms characteristic of spinach-blight, and thus support the conclusion that true spinach-blight and cucumber mosaic are both due to the same virus.

"Inheritability" of the Cucumber-mosaic Virus by Progeny of Infective Aphids. Having concluded that the original spinach-blight is probably identical with cucumber mosaic on spinach, it seemed desirable to make some test of the capacity of the aphid vectors of cucumber mosaic for direct transmission of the virus from one generation of insects to the next in some such manner as had been done for spinach-blight.

McClintock and Smith (13) performed 2 types of experiment in this connection. In the first type, colonies of *Myzus persicae* and *Macrosiphum solanifolii,* originating from viruliferous stock, were bred through several generations on hosts supposedly not susceptible to spinach-blight and were tested at intervals for infectivity by transfers to healthy spinach. In the second type, young of infective *M. solanifolii* were transferred at birth to healthy spinach before they had time to feed upon the mosaic host.

Since the writer has not been able to discover any host plant suitable for colonization of either species of aphid that is not susceptible to infection with cucumber mosaic, either locally or systemically, it was decided to repeat the second type of experiment, using *Macrosiphum solanifolii* as the test species. The following procedure was adopted. Colonies of the aphid were bred for several generations exclusively on cucumber-mosaic spinach of the variety Bloomsdale before being tested in the experiment. All plants used as mosaic hosts showed characteristic symptoms of the disease in moderately advanced stages of infection. Young of adult aphids born and reared on such plants were then removed at birth by means of a fine camel's-hair brush and placed on healthy spinach plants in insect-proof cages. While McClintock and Smith transferred on an average 2 aphids to each plant, it seemed preferable in this test to use a larger number per plant, since previous experience with the transmission of the same virus by the peach aphid had indicated that infection was not regularly obtained with such small numbers of insects.[3] Ten nymphs were, therefore, transferred to each of 15 plants, so that in all 150 young were tested for infectivity. The aphids were closely observed after transference in order to ascertain whether they were in any way injured by handling, any individuals showing

[3] Hoggan, Ismé A. Some factors involved in aphid transmission of the cucumber-mosaic virus on tobacco. (In manuscript.)

any signs of injury being promptly replaced. The aphids were allowed to remain on the plants for a week or more, during which time they made apparently normal development. They were then destroyed by fumigation and the plants observed for signs of infection. As a test of the infectivity of the parent aphids, 10 adults were transferred to each of 15 plants and treated in a similar manner. Fifteen untreated spinach plants served as a control. All plants were observed over a period of 5 weeks before final records were made. Of the 15 plants to which the young nymphs had been transferred, all remained perfectly healthy in appearance throughout the experiment, while of the 15 plants infested with adult nymphs, 8 developed typical symptoms of the disease in less than 3 weeks. Although this does not represent a particularly high percentage of infection, it is approximately what would be expected on the basis of previous results obtained on the transmission of the cucumber-mosaic virus by varying numbers of peach aphid.[4] All control plants remained healthy throughout. There was, therefore, no evidence in this experiment of any direct transmission of the virus from infective parent aphids to their progeny.

The test was not repeated with *Myzus persicae* since a similar experiment, shortly to be reported elsewhere,[5] has been carried out with this species, using cucumber-mosaic tobacco as host. It may be stated, however, that here again there was no indication of any inheritance of the virus by progeny of infective parents.

Properties of the Sugar-beet-Mosaic Virus. Since an effective method of artificial transmission of sugar-beet mosaic was available in a modification of Holmes's rubbing method of inoculation (9), it was thought that a determination of some of the properties of the virus in mosaic-sugar-beet extract might be of value as an aid to later identification. The following properties were studied: longevity *in vitro,* tolerance to dilution, and thermal death point.

All virus extracts were prepared by macerating the leaves of mosaic sugar-beet plants in a meat grinder and straining the juice through cheesecloth. Extractions were made soon after characteristic symptoms of the disease had developed on the plants. Inoculations were made to young sugar-beet plants by rubbing 3 leaves of each plant with a piece of cheesecloth dipped in virus extract until the leaves were considerably bruised, usually the 3 lowest leaves exclusive of the cotyledons being selected. The hands were disinfected between each set of inoculations by washing thoroughly with soap and water. Inoculations from untreated extract, which served as a control, were always made last. Four separate determinations were made for each property, 5 plants in each test being inoculated with each sample of extract.

[4] See footnote [3].

[5] See footnote [3].

In the tests of longevity *in vitro,* 2 cc. of virus extract was placed in each of several test-tubes and stored in the dark at an air temperature of about 70° F. The contents of each tube were aged for a different period of time and separate inoculations made from each. For determinations of the thermal death point, 2 cc. of freshly extracted juice was heated for 10 minutes in a thin-walled test-tube in a water-bath maintained at the desired temperature, then cooled rapidly in cold running water. Dilutions were made by adding 5 cc. of freshly extracted juice to 45 cc. of distilled water, then transferring 5 cc. of the mixture to 45 cc. of distilled water, and so on. In this way dilutions of 1 in 10, 1 in 100, etc., were obtained.

The results of trials of longevity *in vitro* are given in table 4. While

TABLE 4.—*Longevity in vitro of the sugar-beet-mosaic virus*

Age of extract in hours	Number of plants infected of 5 inoculated				Total number of infections (20 plants)
	Trial 1	Trial 2	Trial 3	Trial 4	
0 (fresh extract)	5	5	5	5	20
3	5	3	4	3	15
6	4	2	4	2	12
9	3	3	4	2	12
12	1	3	4	1	9
18	1	2	3	1	7
24	0	1	1	0	2
48	0	0	0	0	0
Uninoculated controls	0	0	0	0	0

some variation occurred between individual trials, it will be seen that the percentage of infection gradually decreased with aging, until after 24 hours the extracts yielded only 2 infections on a total of 20 plants. Extract aged for 48 hours gave no infection in any trial. According to these determinations, therefore, the longevity of this virus *in vitro,* at a temperature of about 70° F., should be placed at 24–48 hours.

The tests of tolerance to dilution (Table 5) yielded consistent results. In these tests there was a marked drop in the percentage of infection secured at a dilution of 1 in 100, and extracts diluted to 1 in 1,000 gave only 2 infections on a total of 20 plants. At higher dilutions, no infection was obtained. The tolerance to dilution was, therefore, concluded to be 1 in 1,000.

TABLE 5.—*Tolerance to dilution of the sugar-beet-mosaic virus*

Dilution of extract	Number of plants infected of 5 inoculated				Total number of infections (20 plants)
	Trial 1	Trial 2	Trial 3	Trial 4	
None	5	5	5	5	20
1 in 10	4	3	4	4	15
1 in 100	1	1	1	1	4
1 in 1,000	0	1	1	0	2
1 in 10,000	0	0	0	0	0
Uninoculated controls	0	0	0	0	0

A preliminary test of the thermal death point of the virus indicated that this probably lay somewhere between 50 and 60° C. for a 10-minute exposure. Tests were, therefore, run at 50, 55, and 60° C., and a separate extraction of juice was made for each exposure in order to avoid any effect of aging, since the virus had already been shown to lose some of its infectivity within a few hours *in vitro* (Table 4). As a control, unheated extract was allowed to stand for 10 minutes at room temperature before inoculation, and this proved to be highly infectious in all trials.

The results of 4 separate experiments are presented in table 6. In each test extract heated at 50 and 55° C. yielded some infection, while extract

TABLE 6.—*Thermal death point of the sugar-beet-mosaic virus (10-minute exposure)*

Temperature in °C.	Number of plants infected of 5 inoculated				Total number of infections (20 plants)
	Trial 1	Trial 2	Trial 3	Trial 4	
50	5	2	3	2	12
55	1	1	2	2	6
60	0	0	0	0	0
Unheated extract	5	4	5	5	19
Uninoculated controls	0	0	0	0	0

heated at 60° C. gave none. The thermal death point of the virus was, therefore, concluded to be between 55 and 60° C.

DISCUSSION

Evidence has been presented above pointing to the conclusion that the disease originally described as "spinach-blight" is identical with cucumber mosaic on spinach. The wide range of hosts of the cucumber-mosaic virus and the cultivation of several of these in some of the spinach-growing districts of the United States, together with the ready transmission of the virus by various species of aphids, would no doubt be sufficient to account for the epidemics of the disease that occurred in certain regions before the introduction of resistant varieties of spinach. It is unfortunate, however, that direct comparisons have not been possible with spinach-blight from districts where this disease is, or has been, most prevalent. Final test of relationship should rest upon a relatively simple matter of inoculation from affected plants to one or more characteristic hosts of the cucumber-mosaic virus; though admittedly there might still be insufficient foundation for the assumption that a disease now recognized as spinach-blight was actually identical with the trouble with which McClintock and Smith dealt 15 years ago. The demonstration that spinach is susceptible to several distinct viruses having certain characteristics in common suggests the possibility that more than one virus disease of this host may have been included at one time or another under the name spinach-blight, although it appears most likely that the disease originally described by McClintock and Smith (13) was strictly due to a single virus. L. K. Jones (11) reports the occurrence of beet mosaic on spinach in Washington, and it is possible that the crop may be subject to different virus diseases in different parts of the country. Moreover, if the conclusions reached in the present paper are correct, the spinach mosaic described in Germany would appear to be due to the same virus as sugar-beet mosaic, as originally suggested by Böning (2), but not to be identical with the spinach-blight of McClintock and Smith. Such considerations emphasize again the need of a critical analysis of the specific virus or viruses concerned in any particular disease, and the value of determinations of differential characters other than symptoms as additional means of diagnosis.

If one accepts the conclusion that the original spinach-blight and cucumber mosaic on spinach are identical, another interpretation might be applied to certain results obtained by McClintock and Smith, believed to indicate a direct transmission of the virus from one generation of aphids to another. Cucumber mosaic is now known to have a wide host range and to affect such plants as potato, eggplant, and pepper, hosts used by these investigators for the breeding of successive generations of aphids from infective parents. Certain other hosts from which the aphids were tested, namely, lettuce, celery, and mustard, have also been found by the writer to be susceptible

to this disease. Although McClintock and Smith obtained no infection of spinach by needle inoculation from several of the hosts upon which the aphids had bred, in the light of present knowledge it seems possible that such plants may actually have served as sources of virus for successive generations of aphids. Furthermore, no evidence has been obtained in connection with the cucumber-mosaic virus that the young of infective parent aphids are themselves infective before they have fed on a mosaic host. In view of the great fundamental significance attaching to any fully authenticated case of direct inheritance of a plant virus by progeny of an infective insect vector, it is, therefore, felt that particular caution should be exercised in accepting such an interpretation of results in the absence of further confirmation.

Other points of interest that have arisen during the investigation may barely be touched upon at the present time. The curious behavior of the cucumber-mosaic virus on sugar beet resulting in systemic or only localized infection according to the mode of introduction of the virus into the plant suggests specific relationships with the various tissues of the host about which much remains to be learned. The differential behavior of the cucumber- and sugar-beet-mosaic viruses with respect to the development of local or systemic infection both on sugar beet and on tobacco might be utilized as a means of separation of either virus from a combination of the two. Attention is directed also to the fact that, while previous studies on "local lesions" have dealt mainly with those induced by artificial inoculation, the present investigation affords examples of similar lesions resulting from infection by aphids and representing feeding points of viruliferous insects.

SUMMARY

Spinach, *Spinacia oleracea,* is shown to be susceptible to at least 3 different virus diseases, all of which characteristically cause conspicuous yellowing and necrosis of the foliage, while 2 of them frequently result in death of the entire plant. These diseases are: cucumber mosaic, sugar-beet mosaic, and tobacco ring-spot. That each of these diseases is due to a distinct, specific virus is concluded from a comparison of their symptoms and behavior on certain common hosts.

Cucumber mosaic is shown to be transmissible to sugar beet, systemic or localized infection resulting according to the mode of transmission employed. The development of local lesions as a result of infection by aphids is described in the case of cucumber mosaic on sugar and of sugar-beet mosaic on tobacco.

Cucumber mosaic is very readily transmissible to spinach (var. Bloomsdale) by artificial inoculation and by the aphids *Myzus persicae* and *Macrosiphum solanifolii.* The symptom sequence on this host closely resembles

that of spinach-blight as originally described by McClintock and Smith. Virginia Savoy spinach, known to be resistant to spinach-blight, shows definite indications of resistance to cucumber mosaic under greenhouse conditions.

Spinach plants obtained from Long Island, said to be naturally infected with spinach-blight, were shown to be infected with the virus of cucumber mosaic (*cucumber virus 1*).

Sugar-beet mosaic is readily transmissible to spinach by means of aphids and by artificial inoculation, the Virginia Savoy variety showing no indications of resistance to the disease under greenhouse conditions. The symptoms on spinach differ in several respects from those described for spinach-blight, although closely resembling those of a mosaic of spinach present in Germany.

Tobacco ring-spot is readily transmissible to either variety of spinach by artificial inoculation, though the resulting symptoms bear no very close resemblance to those of spinach-blight. No transmission of the virus was obtained by means of aphids.

Determination of certain properties of the sugar-beet-mosaic virus, made as an aid to later identification, indicates that the longevity *in vitro* is between 24 and 48 hours at a temperature of about 70° F., the tolerance to dilution 1 in 1,000, and the thermal death point for a 10-minute exposure between 55 and 60° C.

On the basis of the extreme similarity in symptom sequence between cucumber mosaic on spinach and the spinach-blight of McClintock and Smith, together with their ready transmission by artificial inoculation and by aphids, and the resistance to either disease manifested by the Virginia Savoy variety, it is suggested that the 2 diseases are identical. On the other hand, a mosaic of spinach described in Germany and considered by some to be identical with the American spinach-blight is believed to be due to the virus of sugar-beet mosaic.

No indication was obtained of any direct transmission of the cucumber-mosaic virus from infective parent aphids, *Macrosiphum solanifolii*, to their progeny. The evidence previously presented as indicating an inheritance of the spinach-blight virus by successive generations of aphids is discussed in the light of the possible relationship between this disease and cucumber mosaic, and reasons are advanced for not accepting this evidence as conclusive in the absence of further confirmation.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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RECORDS OF CITRUS SCAB MAINLY FROM HERBARIUM SPECIMENS OF THE GENUS CITRUS IN ENGLAND AND THE UNITED STATES

ANNA E. JENKINS AND H. S. FAWCETT[1]

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INTRODUCTION

For the purposes of obtaining more information on the occurrence and distribution of the disease known as citrus scab, material of the genus Citrus (mostly cultivated) was examined in 1930 at the herbaria of the Royal Botanic Gardens, Kew, and the Department of Botany, British Museum, South Kensington, London, England, and in 1931, at the Gray Herbarium, and Arnold Arboretum, Harvard University, Cambridge, Massachusetts; New York Botanical Garden, New York, N. Y.; the Philadelphia Academy of Natural Sciences, Philadelphia, Pennsylvania, and the United States National Herbarium, Washington, D. C.

Tabulations and discussions of data obtained from this study are here presented, since it is thought that they may be helpful in tracing some of the history and distribution of the disease. A few supplementary data, based mainly on mycological specimens, are included.

HISTORICAL REVIEW OF THE DISEASE

It is generally supposed that the Sphaceloma pathogen of citrus scab, known in the United States as *S. fawcettii* Jenkins, is indigenous to the Orient, where its suscept (Citrus) is (20) also native. According to Tanaka (19), the earliest literature record of the Japanese name of citrus scab is in an unpublished manuscript written in 1818 by Yoshiyuki Murase. This is also the earliest known record of the disease. Other early records in the East are Nagasaki, Japan, 1863 (11), and Queensland, Australia, 1876 (21, p. 4). The Nagasaki record is based (11) on phanerogamic specimens showing scab lesions, in the herbarium of the Hong Kong Botanical Gardens.

In the Western Hemisphere, early North American records of the disease are Florida, 1878 (9), about 1884 (18), and about 1885 (14); and early South American records (on other than the common sweet orange (*Citrus sinensis* Osbeck), Argentina (2) and Paraguay (15), 1920, and Brazil, 1923 (22). Swingle and Webber (18) believed that scab was introduced into Florida on Satsuma (*C. nobilis unshiu* Sw.). If so, such introduction must have taken place between 1876, when Satsuma was first brought to

[1] Part of a paper read at the annual meeting of The American Phytopathological Society, December 30, 1931 (10). The remainder of the paper, dealing with citrus canker, will be presented separately. Thanks are due to A. D. Cotton, W. R. Maxon, C. E. Kobuski, F. W. Pennell, J. Ramsbottom, E. M. Wakefield, and A. C. Weatherby, for courtesies in connection with the examination of the phanerogamic specimens, and to H. R. Fulton and W. T. Swingle for a critical reading of this paper.

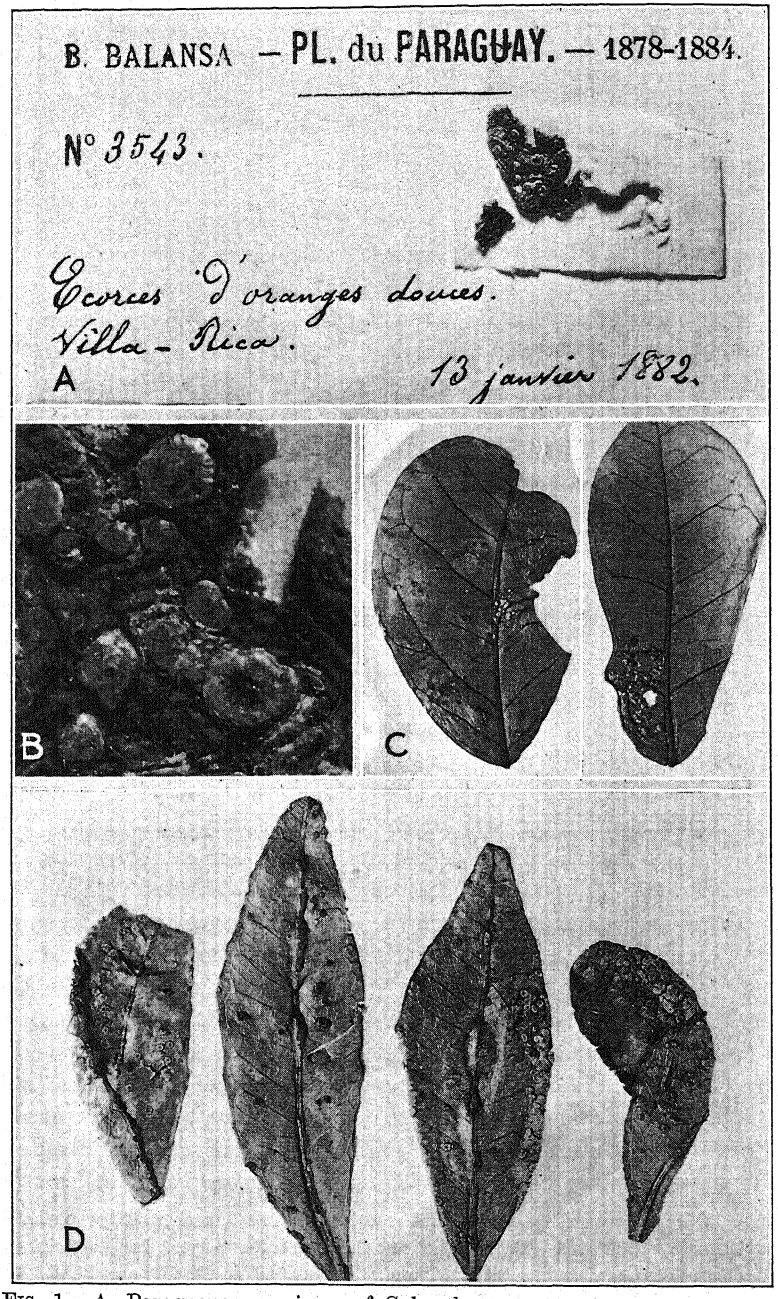


FIG. 1. A. Paraguayan specimen of Sphaceloma on sweet orange, together with label. ×1. B. Enlargement of A. ×7. C. Scab on leaves of *Citrus aurantium*, Nagasaki, Japan, 1863. ×1. D. Scab on leaves of *Citrus* sp. from Chosen, 1906. ×1. Photographs by M. L. F. Foubert.

TABLE 1.—*Identification of citrus scab on phanerogamic specimens of the genus Citrus in herbaria in England in 1930 and in the United States in 1931*

Herbarium[a]	Data on specimens				Name of collection, collector, and number on specimen, where given	Identification
	Date of collection	Place of collection	Kind of citrus: Tanaka's identification, where given	Kind of citrus: Name on label		
Herb. Kew	As early as 1840[b]	Java	*C. limonia* Osbeck	*C. amara* Hassk.	Plantae Bogorienses exsiccatae nova vel minus cognitae H. C. Hochrentiner 107	Apparently scab on leaves and fruit
"	Earlier than 1867[c]	India, Bengal	*C. medica* L.	*C. medica*	Hort. Bot. Calcutta	Apparently affected by scab
"	1862	Japan, Nagasaki	*C. nobilis* Lour.	*Citrus* sp.	Herb. Hook. 1867, Herb. of Royal Gardens, Kew, 134. Coll. R. Oldham	Much scab on leaves and fruit
"	"	"	*C. unshiu* Marcovitsch	*Citrus* sp.	Herb. Hook. 1867. No. 642	Much scab on leaves
Gray Herb.	"	"	*C. aurantium* L.	*Citrus* sp.	Royal Gardens, Kew, 601. Coll. R. Oldham	Typical scab lesions on leaves
Herb. Kew, Gray Herb., and U. S. N. H.	1863	"	*C. unshiu* Marcovitsch	*C. nobilis* Lour.	Ex Herb. horti. bot. Petropolitani Maximowicz Iter secundum Rec'd XII 1885	Typical scab leaf lesions, also funnel-like protuberances characteristic of scab
Herb. B. M., and U. S. N. H.	"	"	*C. aurantium* L.	*C. aurantium* L. var. *bigaradia*	Ex Herb. horti. bot. Petropolitani Maximowicz Iter secundum	Typical scab lesions on leaves (Fig. 1, C)
Herb. B. M.	"	"	*C. aurantium* L.	*C. bigaradia* Duby. var. *japonica*	" "	Much scab on both younger and older leaves
Herb. B. M., and Herb. Kew	"	"	*C. aurantium* L.	*C. bigaradia* Duby.	" "	Apparently affected by scab
Herb. B. M.	1868	India, Bengal	*Citrus medica* L.	*C. limonum* Risso.	Coll. C. B. Clarke	One leaf apparently affected by scab

TABLE 1—(*Continued*)

Herbarium[a]	Data on specimens				Name of collection, collector, and number on specimen, where given	Identification
	Date of collection	Place of collection	Kind of citrus: Tanaka's identification, where given	Kind of citrus: Name on label		
Herb. B. M., and A. A.	1906	Japan, Chosen (Korea)	*Citrus* sp. indet.[d]	*Citrus* sp.	Plantae coreaeae leg. U. Faurie 482[d]	Much scab on leaves (Fig. 1, D)
Herb. B. M.	"	"	*C. tachibunda*	*Citrus* sp.	Plantae coreaeae leg. U. Faurie	Typical scab lesions on leaves
Herb. Kew	1911	"	*C. deliciosa*		Plantae coreaeae	Typical scab lesions
A. A.	1914	Japan, Kogoshima	*C. deliciosa* Kniokuru	*Citrus* sp.	Arnold Arboretum, Exp. to Japan 1914. Coll. E. H. Wilson	Typical scab on leaves
Herb. Kew	"	Japan, Kyushu	*C. tangerina* Hort.	*Citrus* sp.	Arnold Arboretum, Exp. to Japan 1914, No. 6284. Coll. E. H. Wilson	Much scab on leaves
"	"	"	*C. sinensis* Osbeck[e]	*Citrus* sp. "Napura"	Exp. Japan 1914, No. 6825	Much scab
"	1918	Japan, Formosa	*C. tankan* Hayata	*Citrus* sp.	Arnold Arboretum, Exp. to Eastern Asia, 1917–1918. No. 10011. Coll. E. H. Wilson	Typical scab
"	"	"	*C. maxima* (Burm. f.) Merr.		Coll. E. H. Wilson	Typical lessions on leaves
A. A.	1928	China, New Territory		*C. aurantium* ?	Herb. Coll. Agr., Sun Yatsen, Univ. F. No. 624. Coll. W. Y. Chum	Typical lesions on leaves

[a] Herb. Kew = The Herbarium, Royal Botanic Gardens, Kew; Herb. B. M. = Herbarium, Department of Botany, British Museum; Gray Herb. = Gray Herbarium; A. A. = Arnold Arboretum; U. S. N. H. = United States National Herbarium.

[b] The date August 21, 1840, appears at the close of Hasskarl's (7) article in which this specimen is cited.

[c] 1867 = Date this specimen (undated) was given to the Herbarium at Kew.

[d] U. Faurie 482 is identified by Swingle (17) as *Citrus nobilis* var. *deliciosa* Sw. n. comb.

[e] Apparently nearer sour orange (*Citrus aurantium* L.) than *C. sinensis*. H. S. F.

Florida (1), and 1878, the earliest date for the occurrence of the disease there (9).

When the disease was discovered in South America, in 1920, it was thought (2, 15) that it was of recent foreign origin there, although G. L. Fawcett (2) suggested that it might have been present in the country earlier without having been reported. Spegazzini (15) believed that the pathogen had been introduced from Florida. Hauman (8), however, considered that the disease differed in some respects from Massee's (12) description of it, which pertains only to the disease in Florida.

The common sweet orange is generally regarded as immune from or highly resistant to attack by the citrus-scab pathogen. Scab of sweet orange is reported, however, in Paraguay (6, 15), Uruguay (6), and Argentina (3). The mycological specimen collected in Paraguay in 1882 (9) is illustrated in figure 1, A and B. G. L. Fawcett (3), in reporting scab of the common sweet orange in Argentina, says that it is distinct from common citrus scab, affecting sour orange (*Citrus aurantium* Osbeck) and mandarin (*C. nobilis deliciosa* Sw.) only slightly, and being practically limited to fruits. This author[2] has recently said that the disease, which was present in Argentina long before the common scab was introduced, causes a dwarfing of young sweet-orange fruits (less than half grown) and greatly disfigures full-grown fruits. Furthermore, in nonsprayed orchards, of which there are many in Argentina, the disease does not extend through the plantation, but affects only about 5 to 10 per cent of the trees. The name sweet-orange scab is here suggested for this disease.

DATA OBTAINED MAINLY FROM PHANEROGAMIC SPECIMENS

Table 1 lists the phanerogamic specimens on which citrus scab, or what appears to be this disease, was found. It will be noted that all of the material is eastern and that the kinds of citrus represented are those recognized as moderately or highly susceptible to scab. As would be expected, none of the specimens are from the Philippines, where the disease is not known to occur. The diseased phanerogamic specimen from Chosen, dated 1906, showing numerous scab lesions, is probably one of the earliest records if not the first record of the disease in that country. Leaves from the specimen in the Arnold Arboretum are shown in figure 1, D. Assuming that the lesions on the two specimens from Bengal are definitely of scab, these now constitute the earliest known record of the disease in India. The Javanese specimen is the earliest available record of the disease outside of Japan. The only other record from Java is dated 1919 (9).

The prevalence of scab at Nagasaki, Japan, in 1862–1863, on several different kinds of citrus is indicated by the several specimens from Nagasaki listed in the table. Leaves from the specimen in the United States National

[2] Letter dated Dec. 20, 1931.

Herbarium, dated 1863, are shown in figure 1, C. The specimen, originally identified as *Citrus nobilis,* dated 1863, is evidently of the same collection as that in the Hong Kong Botanical Gardens, referred to in the previous section. The specimens dated 1862 now supersede this collection as the earliest specimen record of the disease and the earliest record of its occurrence at Nagasaki.

Among the early specimens from the Western Hemisphere examined were those from the British Museum, *i.e.,* sour orange (highly susceptible to scab), from Jamaica (in the Sloane Herbarium) dated 1783, and from the region of the St. John's River, Volusia County, Florida, dated 1848. Upon discussing with Dr. Swingle the fact that this material is scab-free, as is (9) that in the Schweinitz Herbarium, acquired by the Academy of Natural Sciences, Philadelphia, in 1834, also including sour orange, he contributed the information that as late as 1893, when scab was wide-spread in Florida, he found a grove of naturalized sour orange there that was still unaffected. This was in Lake County, on the right bank of Alexander Spring Creek, near its junction with the St. John's River and a few miles south of Dexter's Lake.

Although not found on South American phanerogamic specimens of citrus examined, citrus scab is present on a number of mycological specimens recently received from that continent. The kind of citrus represented and the source of this material are as follows: from Argentina, lemon (*Citrus limonia*) collected in 1925 by G. L. Fawcett and common sweet orange collected in 1927 by Max Kisliuk; and from Brazil, common sweet orange, lemon, and lime (*C. aurantifolia* Sw.) intercepted in 1930–31, by inspectors of the United States Bureau of Plant Quarantine; Satsuma from the Sub-Estacao Experimental de Limeira, Cordeiro (near Limeira), São Paulo, collected in 1931 by Carlos Wright and sent by E. E. Honey, and Satsuma, lemon (Genoa, rough, and sweet), bitter-sweet orange (*C. aurantium* L.), and lime (Rangpur), collected in 1932 at Vicosa, Minas Geraes, by P. H. Rolfs.

The present study gives added support to the idea that scab originated in the Orient at an early date and was introduced into North America from this region. But it affords no explanation of the origin of the form or forms affecting the sweet orange in South America, in one instance known to have been present as early as 1882. This is only 4 years later than the earliest date for scab in the Western Hemisphere and long before scab was found on other kinds of citrus in South America or on sweet orange (16) in North America, *i.e.,* Florida. The investigation adds no new countries to the known distribution of scab on any kind of citrus, unless there is no previous record from Chosen. The combined literature reports of the disease as now available, however, show that its known world range is considerably

greater than in 1921 (13), 1923 (22), or as late as 1926 (5). This range is as follows:

Eastern Hemisphere: Chosen (Korea), Japan, Formosa, China, Siam, India, Ceylon, Malay Peninsula, Java, Oceania (Bonin Islands, Fiji Islands, Guam, and Hawaiian Islands), Australia (Queensland, New South Wales, and Victoria), New Zealand, Africa (Belgian Congo, Nyassaland, Rhodesia, Sierra Leone, and Union of South Africa).

Western Hemisphere: United States (Alabama, Florida, Louisiana, Mississippi, and Texas), Bermuda, West Indies (Cuba, Dominican Republic, Jamaica, Lesser Antilles, Porto Rico), Trinidad, Mexico, Canal Zone, South America (Argentina, Brazil, Colombia, Paraguay, and Uruguay).

Citing Fawcett (4), Peltier and Frederich (13), in discussing the effects of weather on the world distribution of citrus scab, give 23° C. as the maximal temperature at which infection by the scab pathogen may occur. But Fawcett (4) actually obtained infection at 24.5° and at 27.5° C. in an experiment so arranged that the infection period was prolonged (experiment with detached leaves floating in water). It would seem that infection at these higher temperatures should also be considered in studies involving the temperature relations of this organism. Also, the longer infection period at these higher temperatures may indicate that at such temperatures, under natural grove conditions, there would be much less chance for infection than at 16°–23° C. but that infection would not be precluded.

SUMMARY

Data obtained mainly from an examination of phanerogamic herbarium specimens of the genus Citrus in some herbaria in England and the United States support the idea that citrus scab originated and was present in the Orient at an early date. The Javanese specimen dated as early as 1840 constitutes the earliest specimen record of the disease. The two specimens from Bengal (one dated 1868, the other thought to be much earlier) are the earliest specimen records from India and the specimen from Chosen (dated 1906) is probably the earliest, if not the first, record of the disease in that country. The Japanese specimens dated 1862 constitute the earliest specimen records of the disease. Based mainly on combined literature records the present known range of citrus scab, including the form (or forms) affecting the common sweet orange, is given. Attention is called to the fact that Peltier and Frederich, in discussing the effects of weather on the world distribution of citrus scab, do not take into consideration that scab has been obtained experimentally at 24.5° and at 27.5° C. (where the infection period was prolonged) as well as at the range of 16° to 23° C.

BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.,
AND CITRUS EXPERIMENT STATION
(UNIVERSITY OF CALIFORNIA),
RIVERSIDE, CALIFORNIA.

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PHYTOPATHOLOGICAL NOTES

Physiologic Specialization of Tilletia tritici on Emmer.—Emmer, commonly known as spelt, is grown as a feed crop in the Dakotas and in adjoining states. Vernal (White Spring) or common emmer, now most commonly grown, was brought to the United States by German-Russian immigrants and grown in the Dakotas prior to 1898. Samples of this variety have since been introduced into this region as Yaroslav emmer.[1]

Although the emmers, *Triticum dicoccum,* as a class, formerly were considered resistant to smut, *Tilletia tritici* (Bjerk.) Wint., is now common and frequently destructive in emmer fields of this region. Head infections of 25 to 50 per cent are not uncommon.

Emmer smut caused by *Tilletia levis* Kuehn has not been observed by the writer, although infections by artificial inoculations have been induced without difficulty.

Four collections of *Tilletia tritici* on emmer from different counties in North Dakota and 146 collections on durum wheat were studied critically

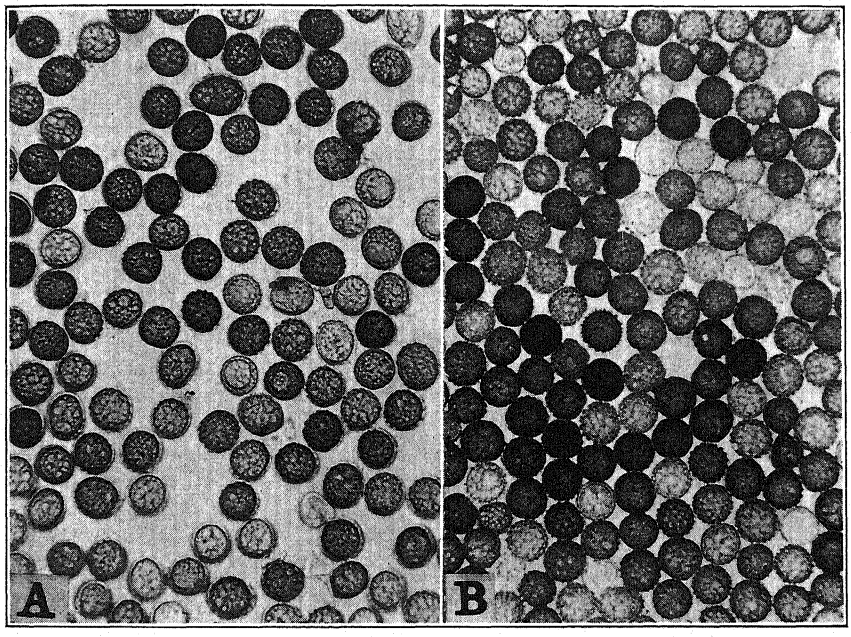


FIG. 1. *Tilletia tritici* from Ceres wheat, developed by artificial inoculations. A. Spores of T408, originating from durum wheat. B. Spores of T394, originating from emmer.

[1] Martin, J. H., and C. E. Leighty. Emmer and spelt. U. S. Dept. Agr. Farmers' Bul. 1429. 1924.

with a microscope. Wide variations were found in the size and shape of spores and in the degree of spore-wall reticulations, but the spores of the 4 collections of emmer smut, T289, T391, T394, and T412, were uniformly unlike these of the durum smuts. They were uniformly of one size, almost spherical and with well-developed reticulations (Fig. 1).

These differences in spores, under certain conditions, were accompanied by a difference in shape of smut balls. This was disclosed by inoculating Ceres wheat.

Single smut balls from each of the emmer collections and from 10 durum collections were each applied separately to 50 seeds of Ceres wheat. The smut balls developing from each inoculation of emmer smut were much elongated, while those from the durum smuts were distinctly rounded (Fig. 2 A). Somewhat similar difference in shape of smut balls from 2 physiologic forms of *Tilletia tritici* was reported by Smith[2] and Holton.[3] In addition to these differences the smut collections were pathogenetically unlike.

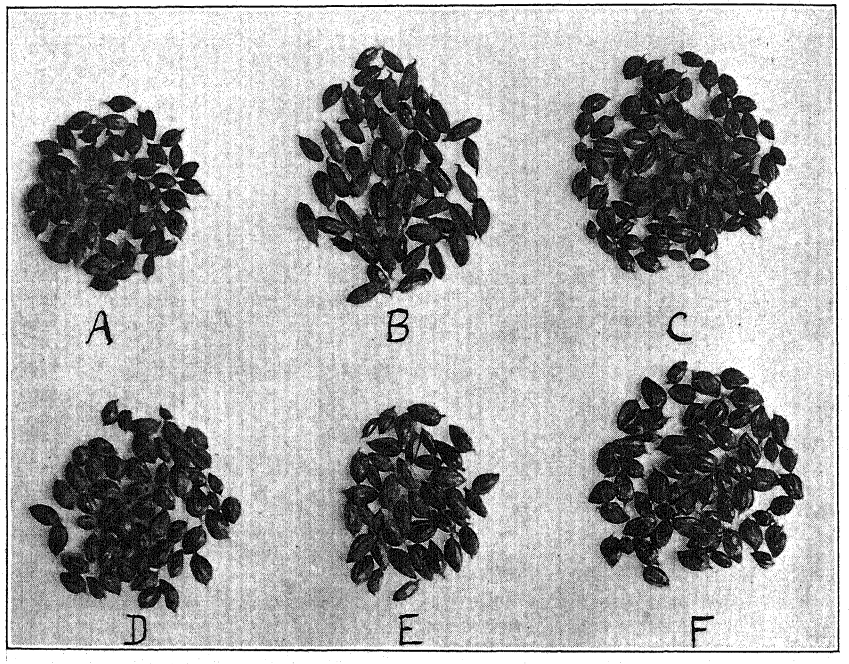


FIG. 2. *Tilletia tritici* from Ceres wheat, developed by artificial inoculations. A, C, D, and F. Smut balls, T401, T402, T405, and T411, originating from durum wheat. B and E. Smut balls, T391 and 394, originating from emmer.

[2] Smith, W. K. Reaction of Martin wheat to three physiologic forms of *Tilletia tritici*. Phytopath. 22: 847–850. 1932.

[3] Holton, C. S. Differences in size and shape of smut balls of two physiologic forms of *Tilletia tritici*. (Abst.) Phytopath. 23: 16. 1933.

In the inoculations of Ceres referred to above the 4 emmer collections produced an average of 24 per cent of smutted heads, whereas the 10 durum smuts produced an average of 86 per cent. In field inoculations, involving triplicated 5-foot rows, the emmer smuts produced low percentages of infection on 10 wheats and relatively high infections on Yaroslav emmer. The 10 durum smuts, conversely, produced relatively high infections on 10 wheats and low infections on Yaroslav emmer.

The collections of emmer smut from the 4 different fields appear to be of 1 physiologic form. Although it is suggested that the emmer smuts of this region generally may belong to this 1 specialized form of *Tilletia tritici*, it remains to be determined definitely whether or not this is true.—W. E. BRENTZEL, Plant Pathologist, North Dakota Agricultural Experiment Station.

Germination of the Oospores of Pseudoperonospora humuli.—Prompt germination of the oospores of the hop disease, downy mildew, *Pseudoperonospora humuli* (Miy. and Tak.), and infection of hop seedlings with inoculum were obtained in connection with trials to determine the disease resistance of new hop varieties. The only other report of oospore germination was made by Arens.[1] His results are substantiated by those here reported, and the importance of the oospore in the study of this destructive disease is established.

Mildew-infected leaves full of oospores, obtained 2 years before, in the fall of 1930, and kept as dry herbarium specimens in the laboratory, were finely ground with a mortar and pestle. The method of germination suggested by Hiura[2] was used. Briefly, the method was as follows: Moist layers of cotton were placed in the upper and lower halves of Petri dishes and a layer of absorbent paper was crumpled to give a corrugated effect on the lower layer of cotton. A small quantity of the powdered leaf was sprinkled on this paper. The contents of the dish were kept moistened and the dishes in all these trials were maintained at room temperature, about 20° C. Germination was obtained also by soaking the mildew-infected leaves in water for several days.

The work was begun on December 20, 1932, and numerous trials were undertaken and observations made several times daily. In a microscopic preparation made from a culture conducted by the first method described and prepared at 11 a.m., December 27, there was evidence of germination

[1] Arens, K. Untersuchungen über *Pseudoperonospora humuli* (Miyabe u. Takah.), den Erreger der neuen Hopfenkrankheit. Phytopath. Ztschr. 1: 169–193. 1929.

[2] Hiura, Makato. Simple method for the germination of oospores of *Sclerospora graminicola*. Science 72: 95. 1930.

at 9:30 p.m., on the next day, December 28. Two sporangia had formed and were emitting zoospores, but in the last stages, for the zoospores were moving about feebly and soon rounded up. Most of the zoospores had already escaped, for only 7 were observed to come from one of the sporangia and 8 from the other. Immediately 6 young Late Cluster seedlings growing in the greenhouse were inoculated with this zoospore material. On the first day of January, 1933, there was evidence of conidiophores on 4 of the seedlings, and on January 2 there was unmistakable evidence of mildew infection.

On January 1 some evidence of zoospore formation was found in the leaf material, which had been soaking in water. Eight seedlings of the Fungles variety were inoculated on this date, and on January 4 all of the seedlings were found heavily infected with downy mildew. The seedlings died from the effects of the mildew within a few days.

In subsequent work an excellent section of tissue containing numerous oospores was found in the leaf material soaking in water. Germination was plainly observed in these cases. The oospores germinated by means of a germ tube, perhaps twice the diameter of the oospore in length, which rounded up at its outer extremity into a sporangium similar to the conidia formed in the summer. Germination of this sporangium by zoospores was hastened by slightly warming the glass slide with a lighted match. It was difficult to obtain exact counts, but more than 30 zoospores emerged from each sporangium. Eight seedlings of the Fuggles variety were inoculated with this material on January 18, and on January 21 all of them showed unmistakable mildew infection.

Measurements showed that the oospores were approximately 30 μ in diameter. The sporangia measured about 26×31 μ. The zoospores were bean-shape and were 5 to 7 μ in size. The zoospores, after swimming around briskly for about 20 minutes at room temperature, rounded up and came to rest.

Knowledge of oospore germination is not only of value in studying the life history of the fungus but also in controlling the disease. Also, it has been necessary to carry the inoculum on live plants, as this disease is an obligate parasite. Now the problem of carrying the fungus for laboratory and greenhouse study is simplified. For example, the plant breeder may collect oospore material in the fall, germinate the spores when he chooses, and inoculate hop plants. Whether newly formed oospores must go through a dormant period before they are capable of germination is not known. The 2-year-old material used in these trials, however, germinated promptly. Additional studies are being conducted.[3]—E. N. BRESSMAN and R. A.

[3] Published as Technical Paper No. 192 with the approval of the Director of the Oregon Agricultural Experiment Station.

NICHOLS, Oregon State Agricultural College and U. S. Department of Agriculture.

The Spermatia of Flax Rust, Melampsora lini.—*Melampsora lini* (Lk.) Desm. is an autoecious long-cycle rust. It is heterothallic, for an isolated, gametophytic infection cannot proceed to the formation of aecia; it remains haploid. But an infection to which spermatia have been transferred from another infection of opposite sex forms aecia rapidly and discharges aeciospores within 3 or 4 days.

The question arises as to how the spermatia enter and become effective in initiating the sporophyte generation. Although the young flax leaf is tender and the walls of its epidermal cells are relatively thin, few of the rust hyphae of any sort grow out to the surface of the leaf. An intensive search for receptive hyphae reaching the leaf surface between epidermal cells or in stomata gave negative results. The spermogonia offer a feasible avenue of entrance and it is not unlikely that spermatia do grow in through them, but an experiment has proved that spermatia from a mature infection can become effective before the spermogonia of another infection develop. Some other method, therefore, must be available. It would appear that surface hyphae are not essential to fertilization in flax rust.

The extreme minuteness of the spermatia makes difficult a detailed study of their structure and behavior. In infections fixed at short intervals after smearing spermatial exudate on the leaf surface at an infection, the spermatium has been observed to flatten down onto the epidermal wall, secrete minute drops of some substance that may serve to attach it to the wall, and push out a fine beak that penetrates the wall diagonally. There is some evidence that this penetration is effected partly by mechanical force and partly by enzymatic action. The spermatium then enters and moves across the epidermal cell, perhaps carried passively by cytoplasmic streaming. Many die at this stage, perhaps overcome by the defensive forces of the host. There are some indications that a few survive. At the inner wall a spermatium attaches itself, again pushes out a fine beak that passes through the wall and then grows, becoming a hypha within the intercellular spaces.

Cell fusions of paired hyphae are of regular occurrence in the young aecium. This is presumptive evidence that the hyphae of spermatial origin maintain an independent existence up to this point.—RUTH F. ALLEN, Division of Cereal Crops and Diseases, United States Department of Agriculture, cooperating with the Agricultural Experiment Station of the University of California.

A Cork-borer Method for Inoculating Trees.—During the past several seasons the writer has been working with fungus organisms that develop in

the cambial region of true firs (Abies spp.). Various methods were used experimentally to introduce the organisms into the cambium. A cork-borer method of inoculation, suggested by E. P. Meinecke, of the Division of Forest Pathology, has been most satisfactory and is especially adaptable to field conditions.

The method used is as follows: After sterilizing the area of bark where the inoculation is to be made, a $\frac{1}{4}$-inch cork borer is employed to cut through the bark to the xylem. The borer is then carefully withdrawn, containing the bark core in the barrel. The inoculum is deposited in the hole and the core immediately refitted into place by forcing it from the barrel, with the plunger, directly into the hole. The core is secured in place and drying out is prevented by the application of waterproof adhesive tape, which is best wrapped completely around the branch or stem. If the tape is put on in patches it has a tendency to loosen or curl back from the ends and, therefore, seals ineffectively.

This method is particularly useful for trees with relatively thick bark, especially when it is desirable to deposit appreciable amounts of inoculum in the cambial region. When a large number of inoculations are to be made in the field the method as described has the advantages of quickness, simplicity, and effective prevention of desiccation. Serious air contamination is unlikely since the bark core is removed for only an instant while the inoculum is being deposited.—ERNEST WRIGHT, Division of Forest Pathology, U. S. Department of Agriculture, San Francisco, California.

REPORT OF THE TWENTY-FOURTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE ATLANTIC CITY MEETING

The twenty-fourth annual meeting of The American Phytopathological Society was held from December 28 to 30, inclusive. Nearly 200 members were present and 112 papers were presented in 11 sessions. There were joint sessions with Section G, A. A. A. S. (the Julius von Sachs Memorial Program) and with the newly formed Mycological Society of America. The contributions presented at this meeting may be classified according to subject as follows: diseases of miscellaneous crops (2 sessions), 15 papers; virus diseases of tobacco, 10 papers; soil treatment, seed treatments, and sprays, 12 papers; diseases of forest trees, 9 papers; virus diseases, 11 papers; diseases of ornamentals, 11 papers; diseases of cereal crops (2 sessions), 20 papers; diseases of deciduous and small fruits, 13 papers; vegetable diseases, 11 papers.

At the annual conference on extension work in plant pathology (held on December 28 under the chairmanship of R. J. Haskell) about 50 specialists took part in discussions of the following topics: use of lantern slides, film strips, motion pictures, photographic enlargements, colored plates, and specimens with demonstrations and exhibits; use of radio (with demonstration), plant-disease schools and study classes, plant clinics, farm-and-home-week programs, contests and clubs, agricultural trains and plant-disease surveys as a method of teaching.

A session on "The Outlook for Plant Pathology under Present Conditions," under the chairmanship of Professor H. H. Whetzel, considered changes in extension methods to meet changed economic conditions and the effect of present economic conditions on the prevalence of some plant diseases.

On December 28 was held the annual dinner, entertainment being furnished under the auspices of the pathologists of New Jersey, New York, Delaware, Pennsylvania, and Harvard University.

Ten new or little-known plant diseases were reported at this meeting: crown gall on *Carnegiea gigantea* (Michael Levine); a sterile but fungus-like leaf spot on pineapple (Melville T. Cook); a new sclerotial fungus from Texas (D. C. Neal and R. W. Wester); a brooming of *Robinia pseudoacacia* (Carl Hartley and L. W. R. Jackson); a Nectria canker of basswood (D. S. Welch); *Nectria coccinea* on beech (John Ehrlich); fig mosaic in California (Ira J. Condit and Wm. T. Horne); a new necrogenic virus disease of potatoes in California (E. S. Schultz and W. P. Raleigh); a Cytospora canker of Picea (C. J. Gilgut and O. C. Boyd). Other scientific contributions included descriptions of the perfect stages of *Sclerotium gladioli* (F. L. Drayton) and of *S. oryzae* (E. C. Tullis); the production of a fertile saltant of *Helminthosporium sativum* by ultraviolet irradiation (F. J. Greaney and J. E. Machacek); acquired immunity to mosaic and spot necrosis in tobacco (J. M. Birkeland); the toxicity of mercuric chloride-potassium iodide solutions (W. P. Raleigh); the genetics of *Sphacelotheca sorghi* and *S. cruenta* (H. A. Rodenhiser); the pathology of crosses between the loose and covered barley-smut fungi (H. A. Rodenhiser and B. F. Barnes); the occurrence of apple scab on stored fruit (C. O. Bratley); the virus diseases of dahlia (Philip Brierley); Sphaceloma on sweet violets (L. M. Massey, R. P. White, and A. E. Jenkins); a new fruit spot associated with apple measles (Anthony Berg); bacterial cankers of Prunus in California (E. E. Wilson); a tuber rot of potatoes caused by *Botrytis cinerea* (Donald Folsom); and a bacterial blight of broad beans in Louisiana (L. H. Person). Abstracts of the papers on the program of this meeting are printed in the January number of PHYTOPATHOLOGY.

OFFICERS AND REPRESENTATIVES

The following officers and representatives were chosen:

President, J. C. Arthur, Purdue University, LaFayette, Indiana.

Vice President, N. E. Stevens, U. S. Department of Agriculture, Washington, D. C.

Councilor (two years), Carl Hartley, U. S. Department of Agriculture, Washington, D. C.

Associate Editors (three years), Lee M. Hutchins, Fort Valley, Georgia; F. L. Drayton, Central Experimental Farm, Ottawa, Canada; Carl D. La Rue, University of Michigan, Ann Arbor, Michigan, and B. F. Lutman, Burlington, Vermont.

Business Manager (one year), F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

Advertising Manager (one year), R. S. Kirby, Pennsylvania State College, State College, Pa.

Committee on Extension (five years), R. E. Vaughan (to replace C. W. Gregory), College of Agriculture, Madison, Wisconsin.

Representatives on the Council of the A. A. A. S. (one year), N. E. Stevens, U. S. Department of Agriculture, Washington, D. C., and H. S. Cunningham, Long Island Vegetable Research Farm, Riverhead, N. Y.

Member of the Board of Governors Crop Protection Institute (3 years), I. E. Melhus, Iowa State Agricultural College, Ames, Iowa.

Representative on Committee on Nomenclature, Botanical Society of America, J. C. Arthur, Purdue University, LaFayette, Indiana.

The following temporary committees were appointed to serve throughout the meetings:

Auditing Committee, Vera K. Charles and C. R. Orton.

Committee on Elections, Mark Woods, A. B. Hatch, and C. S. Holton.

Committee on Resolutions, E. C. Stakman, R. P. White, and W. B. Tisdale.

REPORT OF THE SECRETARY-TREASURER, 1932

At the close of 1931 the Society had 836 members, including the 48 new members elected at the New Orleans meeting. During 1932 six former members were reinstated and a total of 62 members were lost, 2 through death, 18 by resignation, and 42 who were dropped from the Society for nonpayment of dues. Thus, the total membership now stands at 780. Election of the 47 new applicants for membership will bring this number up to 827, a net loss of 9 for the year. Under adverse economic conditions prevailing during the year it would seem that total membership in the Society has been maintained at a surprisingly high figure.

This is especially true in that no drive has been made for new members. It is suggested that members make every effort to call the advantages of membership to the attention of nonmembers who have an interest in the control of plant diseases. Due to the large number of papers presented for publication in PHYTOPATHOLOGY, it will be necessary in the future to accept satisfactory contributions from members only.

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING DECEMBER 14, 1932

Receipts:

Balance from 1931			$2,651.21
Annual dues: 1931	$ 20.00	($10.00 life)	
1932	1,740.54	(294.53 life)	
1933	2,009.30	(535.17 life)	
1934	1.55		3,771.39

Interest on checking account	21.11	
Donations to Lyman Fund included in checks for dues	4.00	
Sales and special sales included in checks for dues	9.63	
Refund by Secretary-Treasurer, expenses of meeting	4.19	
Cash from B. B. Mundkur for dues in other societies	16.17	
Total receipts		$6,477.70

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY (1932, part 1931)	$2,813.04	
Transferred to Sinking Fund savings account	886.00	
Secretarial work	333.27	
Printing (programs, preliminary programs, abstracts, ballots, etc.)	159.69	
Advance of funds to Secretary-Treasurer for expenses of New Orleans meeting	125.00	
Expenses of entertainment at New Orleans Phytopathological dinner	35.00	
Mimeographing and mailing press releases	33.84	
Advance arrangements trip to Atlantic City by Secretary-Treasurer	21.80	
Chicago conference of Centennial Exhibits Committee	24.57	
Postage and stamped envelopes	42.36	
Telephone and telegrams	8.76	
Supplies and furniture	63.35	
Express on abstracts and on letterhead paper	2.25	
Visitor's chair and desk chair for Dr. Butler	24.72	
Checks returned by bank	25.00	
Federal tax on checks	.24	
Donations transferred to Lyman Fund	4.00	
Sales and special sales transferred to PHYTOPATHOLOGY	9.63	
B. B. Mundkur, dues in other societies	16.00	
Balance on hand	1,849.18	
Total		$6,477.70

SINKING FUND

The Sinking Fund, obtained by deducting $5.00 (formerly $6.00) from each life-sustaining membership payment, is now $7,663.35, of which $5,000.00 is invested in first-mortgage notes. During the year one note for $1,500.00 was paid up. As recommended by the investment committee the Secretary-Treasurer has opened a savings account with the Riggs National Bank of Washington, D. C. In this account was deposited the above-mentioned $1,500.00 and also $886.00 accumulated during 1931 and 1932, giving a total in this account, with interest, of $2,393.35. (The balance of $270.00 is in the checking account of the Society).

Respectfully submitted,

F. C. MEIER, *Secretary-Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1932

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING DECEMBER 14, 1932

Receipts:

Balance for 1931			$1,227.33
Subscriptions: 1930	$ 6.50		
1931	59.99		
1932	2,484.12		
1933	651.91		3,202.52
Sales of back volumes and numbers			202.38
Special half-price sale			673.05
Advertising: 1931	$ 61.56		
1932	537.39		
1933	4.00		602.95
Interest on Sinking Fund			390.00
Principal paid on first-mortgage note			1,500.00
Member subscriptions 1931			25.00
Member subscriptions 1932			2,788.04
Sale of Classics No. 1			2.80
Dues to American Phytopathological Society received with subscriptions			22.50
University of Toronto for share in publishing Forward article			110.00
Palestine Experiment Station for share in cost of reprints			10.00
Total receipts			$10,756.57

Expenditures:

Manufacturing PHYTOPATHOLOGY:			
Vol. XXI, No. 12	$719.02		
Vol. XXI, Index	157.88	$ 876.90	
Vol. XXII, No. 1	621.84		
No. 2	611.36		
No. 3	480.63		
No. 4	769.47		
No. 5	556.60		
No. 6	726.72		
No. 7	347.91		
No. 8	329.17		
No. 9	425.38		
No. 10	506.51		
No. 11	389.57	5,765.16	
Postage		489.21	7,131.27
American Phytopathological Society address list			129.64
Secretarial work for Business Manager			229.85
Expenses of office of Editor in Chief			124.07
Editor in Chief for expenses of New Orleans meeting			60.79

Commission and expenses of former Advertising Manager for 1931	119.45	
Expenses of present Advertising Manager	87.90	
Postage and envelopes	122.15	
Deposited Sinking Fund savings account	1,500.00	
Transferred to American Phytopathological Society for dues	22.50	
Subscriptions refunded	19.85	
Checks returned by bank and collection charge on foreign check	12.50	
Miscellaneous (supplies, reprints, etc.)	12.45	
Federal tax on checks	.36	
Balance on hand	1,183.79	
		$10,756.57

Evidence of the degree to which our Editor in Chief, Dr. H. B. Humphrey, has kept expenses of publication within income is found in the fact that the balance remaining out of 1932 receipts available for publishing the December, 1932, number of PHYTOPATHOLOGY was $527.88. The actual cost of this number was $412.08.

At the end of the calendar year 1931 there were 573 subscribers (nonmembers) to PHYTOPATHOLOGY, including 6 complimentary subscriptions. Fifty-nine (24 from U. S. S. R.) were cancelled for 1932 and during the year 18 were dropped from the list because of failure to maintain their subscriptions. With 40 new subscriptions added during the year the net loss was 37, reducing the subscription list to 536.

Respectfully submitted,

F. C. MEIER, *Business Manager.*

REPORT OF THE EDITOR IN CHIEF

The restrictions placed upon the published output of plant-science journals because of declining revenues have registered their effect also on PHYTOPATHOLOGY. The number of manuscripts submitted during 1932 has, however, furnished no evidence that the general economic depression has been a deterrent to the assembling and preparation of research data for publication. The increasing volume of manuscripts submitted for publication in our journal still bears eloquent testimony to the fact that not a few of the papers fill considerably more space than the substance they present really warrants.

In these times, which call for exercise of the strictest economy, our contributors could do much toward speeding up publication by giving greater care to the preparation of their manuscripts. It is sometimes necessary to return a manuscript to its author two or three times for revision or possible rewriting before it is in acceptable shape for the printer. Many contributors give too little thought to the preparation of tabular data; some of the manuscripts contain tables without headings; others present tables two or more of which could be condensed to a single table. The illustrative matter accompanying the papers published in volume 22 of our journal has, in the main, been more satisfactory than in the past. Some of the illustrations have been exceptionally choice.

The 22nd volume of PHYTOPATHOLOGY contains 1,002 pages of printed matter and illustrations. The preceding volume comprised 1,207 pages of such matter. The 1,002 pages of PHYTOPATHOLOGY for 1932 are classified as follows: seventy-five articles, 30 phytopathological notes, 3 reports, 5 book reviews, 108 abstracts (3 by title only), 1 plate, and 160 text figures. During the period January 1 to December 31, 1932, approximately 157 manuscripts of articles, phytopathological notes, reports, book re-

views, etc., and 136 abstracts (4 by title only) have been submitted. Of this number, 14 manuscripts were returned to their authors for revision, 2 were found unacceptable, and 3 were withdrawn. Of manuscripts submitted in 1931, too late for publication that year, 1 was returned to its author for revision, 2 were not accepted, and 1 was withdrawn. Of the several manuscripts received during the current year, 17 articles, 7 phytopathological notes, and 117 abstracts (2 by title only) are now in press. The index for volume 21 of PHYTOPATHOLOGY was published as a supplement to the February, 1932, number.

The editor wishes to acknowledge with gratitude the highly efficient clerical and editorial service rendered by Frances W. Todd. Thanks are due also to each of the several members of the editorial board and to The Science Press Printing Company for the part each has shared in maintaining the standards of editorial excellence and workmanship of PHYTOPATHOLOGY.

Respectfully submitted,

H. B. HUMPHREY, *Editor in Chief.*

REPORT OF THE ADVERTISING MANAGER

In 1932 a total of 96 advertisements were run in PHYTOPATHOLOGY. These comprised a total of 70⅝ pages. Of the total advertising, the cash advertising consisted of 61 advertisements, occupying 44⅛ pages, valued at $713.05; further, the exchange, PHYTOPATHOLOGY back-number sale, advertising directory, etc., accounted for 35 advertisements, occupying 26½ pages.

The amount and value of the total advertising space contracted in 1932 remained very nearly the same as in 1931; however, PHYTOPATHOLOGY, along with nearly all other journals, was affected by the general business conditions. A total of 19¼ pages of advertisements was lost by cancellation.

An effort has been made during the past year to make PHYTOPATHOLOGY advertising of greater value both to members of the Society and the advertiser. An advertising directory has been included, which lists each advertiser and his products.

The advertising manager wishes to express his appreciation of the valuable assistance and suggestions given by J. F. Adams, the former advertising manager, and to The Science Press Printing Company, publisher of PHYTOPATHOLOGY.

Respectfully submitted,

R. S. KIRBY, *Advertising Manager.*

COMMITTEE OF EDITING PHYTOPATHOLOGICAL ABSTRACTS

That the scientific merit of the abstracts of papers submitted for inclusion in the programs of the annual meetings and for publication in PHYTOPATHOLOGY is still regarded by a considerable proportion of the Society membership as too slight to justify this use of the members' time, the Journal's space, and the Society's funds is well known to the committee on editing abstracts. It is quite patent also that individual members are not disposed to recognize in their own contributions to the Society's abstract problem the same shortcomings as those deplored in the abstracts submitted by others. And some never submit any abstracts at all, so that their proficiency cannot be gauged; but they complain of what other members write, nevertheless. Because the issue of scientific worth of the abstracts is touched with personal interest, the committee still tends to err on the side of toleration. Abstracts are accepted that the committee knows make no significant contribution to our science; the committee is then blamed for low standards and lax enforcement; every year some one proposes that the Society cease printing abstracts, others rise to defend them, "and so, far into the night, the old battle rages."

The real difficulty, as has been pointed out before, is that these abstracts represent a place on the program, as well as the author's conception of a scientific note worthy of publication. The committee on editing abstracts has never received a mandate from the Society to act as a Censor of Programs. It holds, until the Society rules otherwise, that it is the inalienable right of every member to aspire to a place on the program and to submit in fulfillment thereof any title in the field of phytopathology that the Secretary will accept. The substance of the discourse and the manner of presentation are the concern of the individual, subject only to his own regard for his professional standing, the time limit, and the patience of his audience.

Accordingly, this committee does not presume to say what papers may or may not have a place on the program. Our function is construed to be only the reviewing of the abstracts to pass on their scientific merit and novelty, and to edit them when necessary to meet reasonable standards of literary expression. We do not want to enter into a discussion here of the advisability of charging the cost of printing the abstracts against the authors, but we urge the Society to support the abstracts committee, whether this committee flourishes next year under the impetus of a new deal or languishes under its present personnel, in a strict application of the following policy, authority for which was voted by the Society two years ago. Abstracts that do not, in the judgment of the committee, measure up to standards of scientific worth and novelty comparable to those maintained for full-length papers that are printed in PHYTOPATHOLOGY will be published by title only.

Respectfully submitted,

FREEMAN WEISS, *Chairman.*

COMMITTEE ON COOPERATION WITH BIOLOGICAL ABSTRACTS

The editor of BIOLOGICAL ABSTRACTS has proposed that this Society adopt a system, already in effect with several other scientific journals, for more promptly obtaining abstracts of papers published in PHYTOPATHOLOGY. In brief, the system provides for the preparation of abstracts by the author of the paper and sets up a method of distributing requests for abstracts, and in many cases the return of abstracts by authors, simultaneously with the correspondence ordinarily incident to the distribution and return of galley proof.

Consultation with the editorial board of PHYTOPATHOLOGY discloses that the proposed method is acceptable to the editors of our journal and will be given a trial.

According to this system, the editor of BIOLOGICAL ABSTRACTS will furnish the editor of PHYTOPATHOLOGY with a supply of forms for requesting abstracts. One of these requests will be attached to the galley proof of each paper to be published in our journal and will be sent to the author, who will prepare the abstract and return it, with the proof or separately, to the editor of PHYTOPATHOLOGY. The latter will collect all the abstracts that pertain to the original papers, which make up an issue of the journal, and will send them, together with a notice of the probable issue date of this number, to the editor of BIOLOGICAL ABSTRACTS. As soon as this number of the journal is distributed, the editor of BIOLOGICAL ABSTRACTS will send the abstracts pertaining thereto to his assistant editors in charge of the respective sections. Thus, all lapse of time between the appearance of the original paper and the securing and distribution of abstracts to the section editors of BIOLOGICAL ABSTRACTS will be avoided. It is expected that the abstracts may even be published within a month or two of the publication of the original paper.

It is hoped in this way to make BIOLOGICAL ABSTRACTS of real service as a current review of original publications within even a limited field, such as phytopa-

thology, in addition to its service in covering the biological field as a whole and in furnishing an annual subject-matter index thereto.

Respectfully submitted,

FREEMAN WEISS, *Acting for the Chairman.*

REPORT OF COMMITTEE ON INVESTIGATION OF FOREIGN PESTS AND DISEASES

An expedition sent by the Bureau of Plant Industry (Horticulture and Foreign Plant Introduction) to South America discovered potato wart in Bolivia, thus establishing for the first time the presence of this disease in that country, and, incidentally, throwing further doubt upon the European origin of *Synchytrium endobioticum*, since this fungus has been known from Peru since 1929.

A little-known smut of potatoes caused by *Polysaccopsis hieronymi* (Schroet.) P. Henn. was collected in the Andes Mountains near Meridia, Venezuela, by C. E. Chardon and forwarded to Washington for identification. This is the first report of this smut on potato tubers, the former collections from Argentina, Bolivia, and Brazil being recorded as occurring on branches and pedicels of *Solanum* sp. It occurs in Stevenson's list under the name *Urocystis hieronymi*.

The smut disease of Colchicum bulbs was intercepted in 1932 at Washington in shipments from Holland. As high as 12 per cent of the bulbs were infected with *Urocystis colchici* and higher percentages were detected in 1931 shipments.

In view of the present conditions with respect to funds for our investigations in foreign countries, the following suggestions are offered for consideration:

(1) Members of our Society who are making visits to foreign countries might secure valuable information with respect to specific parasites if they would communicate with the committee concerning their itinerary.

(2) Similar visits by mycologists, botanists, horticulturists, and others familiar with pest problems could be made contributory to our knowledge of foreign-pest and disease control. The committee will welcome such cooperation.

(3) In view of the financial difficulties already mentioned, there is another avenue of help, *i.e.*, the assistance of special *attachées* of the Diplomatic Service. Such assistance might be helpful in many ways and should be carefully considered.

C. R. ORTON, *Chairman*,
W. H. MCCUBBIN,
F. C. FROMME.

COMMITTEE ON PRESS

Following the custom of the past 4 years, the press committee placed its duties in the hands of the Press Service of the The American Phytopathological Society, an independent organization established for the purpose of issuing the abstracts of the midwinter meetings in a form suitable for publication in the newspapers of the country.

The abstracts (with the exception of about 5 were rewritten and 50 mimeographed copies released through Austin Clark. In previous years, it will be recalled, between 25 and 30 pages of mimeographed news stories have been released to nearly 500 newspapers and farm magazines. This year it has not been possible to continue this work.

That this work has been profitable and well worth the Society's efforts is evidenced by the response from the public to a story on the use of naphthalene (*cf.*, Weiss & Evinger, PHYTOPATHOLOGY 22: 1932) as a fungicide given wide circulation *via* the NEW YORK TIMES, as well as the accounts published in the MARKET GROWERS JOURNAL wherein nearly all the vegetable papers of last year were reported.

It is hoped that in another year, if this sort of beneficial press agentry be continued, a greater degree of cooperation between the membership of the Society and various institutions may be maintained, so that the material can be in the press committee's hands as soon before November 15 as possible, in order that it may be assembled in its effective form, mimeographed, and mailed not later than December 1.

For his invaluable suggestions this year, as in earlier ones, the committee is indeed grateful to Mr. Don B. Reed, managing editor of the WASHINGTON POST. Mr. Reed, in his capacity as consulting editor of the Press Service, especially last year (1931), was unquestionably responsible for the hearty reception given our material by the press of the country.

W. A. WHITNEY, *Chairman.*

COMMITTEE ON ELECTIONS

The committee on elections, after having opened sealed ballot envelopes and counting ballots for each nominee, reports election of the following officers: President, J. C. Arthur, Vice President, N. E. Stevens, Councilor, F. D. Heald.[1]

MARK WOODS, *Chairman.*
A. B. HATCH,
C. S. HOLTON.

COMMITTEE ON NECROLOGY

During the calendar year 1931, there have been two deaths, as follows:

Prof. Arthur Jaczewski, February 12, 1932.
Dr. Roland Thaxter, April 22, 1932.

A. G. JOHNSON, *Chairman,*
G. P. CLINTON,
M. B. WAITE.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

American Association for the Advancement of Science. A brief, informal report of the representatives on the *Council of the American Association for the Advancement of Science* was presented by D. Reddick.

Auditing Committee. The Auditing Committee appointed December 28, has examined the books of The American Phytopathological Society and PHYTOPATHOLOGY and found them correct.

VERA K. CHARLES, *Chairman,*
C. R. ORTON.

Committee on Extension Work in Plant Pathology. The activities of the committee on extension work have been chiefly along two lines: the publication of the mimeographed leaflet *The Extension Pathologist* and the making of arrangements for the extension conference at the present Atlantic City meeting. Owing to the fact that funds for the mimeographing in the U. S. Department of Agriculture were cut in half this year, only 3 numbers of *The Extension Pathologist* have been issued to date and each number is restricted to a total of 8 mimeographed pages. The committee will appreciate receiving suggestions for improvement and also contributions to this publication.

No summer meetings of the committee or of extension pathologists were arranged in 1932 because of the unsettled economic conditions.

C. T. GREGORY, *Chairman.*

[1] See item under Other Business.

Committee on Century of Progress Exposition. At the request of the Chairman, L. R. Jones, the duties of this office were assumed in 1932 by H. W. Anderson. A meeting of a part of the committee was held at Chicago, where contact was made with J. F. W. Pearson, who has charge of the exhibits on biologic science in the Basic Science Building. The plans of the committee called for financial support from the general funds of the Century of Progress Exposition. According to Dr. Pearson, this support was not possible because of the lack of funds. He gave us to understand that funds for other exhibits were being supplied from commercial sources. It was the opinion of the committee that we were not empowered to go ahead under these conditions, and so reported to the Secretary of the Society.

In correspondence with Secretary Meier it was suggested that the committee request support from the Agricultural Section as represented by A. F. Woods and C. W. Warburton of the United States Department of Agriculture. It seemed from their statements that sufficient financial support could not be secured to warrant a special exhibit, which, in the opinion of Dr. Pearson, would cost in the neighborhood of $5,000.

It is the opinion of the Acting Chairman that your committee should continue to function in an advisory capacity but should make no further effort to secure a separate phytopathological exhibit.

(Dr. Jones requests that H. W. Anderson be made active Chairman of the committee.)

L. R. JONES, *Chairman,*
C. T. GREGORY,
G. K. K. LINK,
M. B. WAITE,
H. W. ANDERSON.

Representatives on Board of Governors, Crop Protection Institute. The Crop Protection Institute continued its productive work during 1932 with as many active research projects as in any other year of its history. The scope of its work is gradually increasing, as more and more industrial organizations entrust it with funds for prosecution of research. Large corporations have reduced their research activities and it has been difficult throughout 1932 to secure financial support of research by industrial funds. Corporations have been interested in arranging for preliminary exploration during this period of reduced business activity. They are interested in searching for new outlets and new products at the present time, so they may be in a position to engage in more comprehensive development later and may have a groundwork laid for such plans. Preliminary exploration of this kind can be done at moderate expense and can be carried along step by step over relatively short periods of obligation.

In 1932 the Institute directed 16 active research projects. Eight of these projects were related to problems in plant pathology, covering such phases as development of new copper sprays and dusts, iodine salts as fungicides, fungicides in combination with oil sprays, combined fungicides and insecticides, industrial adhesive tape to reduce losses from crown gall, plant introduction and improvement, development of flotation sulphur, copper salts in relation to plant nutrition and plant stimulation, etc. The various projects are being conducted through the cooperation of 9 experiment stations, and each project is under the supervision of a committee composed of 3 plant pathologists. Publication of results has been made where conditions warrant publication.

The Board of Governors, as in the past, is composed of 3 members elected by the American Phytopathological Society, 3 members elected by the American Association of Economic Entomologists, and 2 members elected by the Association of Agricultural Chemists. The officers elected by the Boards of Governors for 1933 are W. C. O'Kane,

Chairman, Durham, New Hampshire; J. F. Adams, Vice Chairman, Newark, Delaware, and Paul Moore, Secretary and Treasurer, National Research Council, Washington, D. C.

W. F. MARTIN, *Chairman,*
J. F. ADAMS.

Resolutions Committee. The American Phytopathological Society wishes to express its heartiest thanks to Albert Skean, Executive Secretary of the Atlantic City Convention and Publicity Bureau, for his very efficient help in making such satisfactory arrangements for the meetings of the Society.

The American Phytopathological Society wishes to express its appreciation to the management of The Madison Hotel for extraordinary courtesies and hospitality during the meetings. We wish to thank especially Frank E. Sutch, Publicity and Convention Manager, Eugene C. Fetter, Managing Director, and Linton B. Arnold, for extraordinary helpfulness in connection with our meetings.

The generosity of Domenico Pirone has made it possible to translate and make available to pathologists and mycologists the extremely interesting publication of Fontana on rusts. The American Phytopathological Society wishes, therefore, to express to Mr. Pirone its appreciation of his interest in the progress of our science and its deepest gratitude for his extraordinary generosity. The Society is thankful also to P. P. Pirone for making the translation.

Whereas the committee on the banquet program labored very effectively, possibly even long and arduously, in delineating our individual and collective mental and emotional growth curves, much to our profit and merriment, *BE IT THEREFORE RESOLVED* by The American Phytopathological Society, even after having recovered from the immediate effects of the meteoric shower of pungent wit, that we express our appreciation of the kindly spirit in which the quips were quipped and our thanks for the thought- and mirth-provoking efforts of W. H. Martin, Chairman of the committee, to Richard White, to Mr. Ology, McGinty, and others who helped produce the festival of fun.

Recognizing the fact that the Secretary-Treasurer of the Society and Business Manager of PHYTOPATHOLOGY, F. C. Meier, and the Editor in Chief of PHYTOPATHOLOGY, H. B. Humphrey, work hard and get in return nothing except occasional criticisms and complaints, we wish to record our appreciation of their efficient and unselfish services and to thank them deeply and sincerely for contributing their time and energy to the affairs of the Society.

BE IT RESOLVED that no limitation be placed on the surge of New Year's resolutions to help insure the future financial support for our journal, PHYTOPATHOLOGY, of which we are all proud and of which we wish to become prouder, and that we resolve to transmutate our resolutions into donations, in order that we may help the present generation and future generations to publish promptly and effectively the results of pathological researches.

E. C. STAKMAN, *Chairman,*
W. B. TISDALE,
RICHARD WHITE.

ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report, the Council submitted the following actions, which were approved by the Society.

1. On consideration of an offer from H. H. Whetzel to personally handle for the Society business management of Phytopathological Classics, with editorial work by H. B. Humphrey, it is recommended that this offer be accepted with the provision that the arrangement may be terminated at the will of either party on 1 year's notice, the money on hand and remaining stock to be returned to the Society.

2. It is recommended that, in conjunction with the general meeting of the American Association for the Advancement of Science and associated societies, a combined Chicago and summer field meeting be scheduled during the time set by the American Association for the Advancement of Science and that the President advise with the Council regarding appointment of a committee to handle arrangements.

3. It is recommended that enrollment of new life members be temporarily discontinued, pending study by the committee on investments of the financial return from this class of members, the committee, if necessary, to increase the rate for new members in this class.

4. On consideration of the excellent service to the Society rendered by W. A. Whitney, who, for several years, has served as press representative, the Council passed a resolution commending Mr. Whitney for this work that urged him to reconsider his resignation from these duties. The Council instructed the Secretary to report on Mr. Whitney's work in some detail to the Society in business session.

5. That, as was the case in 1932, the Editor in Chief of PHYTOPATHOLOGY be allowed actual expenses for 1933 up to or within $300.00 for secretarial and editorial assistance.

OTHER BUSINESS

The reports of officers, committees, and representatives, as printed above, were accepted.

The Secretary read the following from a telegram from President F. D. Heald—"Prevented from coming to Atlantic City by influenza. Greatly disappointed as I had been looking forward to the trip and the pleasure of meeting many old friends and making new acquaintances. Best wishes for an enjoyable and successful meeting." Following this reading, the Society instructed the Resolutions Committee to prepare a telegram to be sent by the Secretary expressing regret of those present in Atlantic City at President Heald's enforced absence.

It was moved and carried that in view of the fact that under the Constitution the retiring President, F. D. Heald, automatically retains a seat in the Council for 1933, Carl Hartley, who received the second largest number of votes in the mail ballot, be elected Councilor for 2 years.

Resignation of L. R. Jones from the Committee on Exhibits at the Chicago Century of Progress Exposition and his recommendation that H. W. Anderson be assigned the chairmanship of this committee were approved.

Attention was called by the Secretary to good results obtained from activities of press representative W. A. Whitney and methods used in making his popular rewrites of abstracts available to the press.

Proposal submitted in a telegram by W. A. Whitney to the effect that the annual meetings in 1933 be devoted to a Silver Anniversary and Memorial Program was referred to the Council with power to act.

Motion made by J. C. Walker that the Council, in consultation with the Editor in Chief and the Business Manager of PHYTOPATHOLOGY, be empowered to charge authors a set price per page for articles published in PHYTOPATHOLOGY, said price to be determined by the Council, provided said price does not exceed $1.00 per page, was carried.

Motion introduced by A. G. Johnson that publication of abstracts be abolished was defeated after much discussion.

It was moved by L. R. Jones and carried that the Council be empowered, in the case of abstracts, to charge up to full cost of publication, removing the limit of $1.00 per page previously authorized for publications in PHYTOPATHOLOGY.

ARTHUR JACZEWSKI

NOVEMBER 3, 1863–FEBRUARY 12, 1932

Arthur Jaczewski was privately educated in Russia and took advanced studies in the University of Berne, Switzerland. He also attended special lectures at the Sorbonne and the universities of Lausanne and Montpellier.

In his earlier years he botanized extensively, privately, in Russia, Switzerland, Italy, and Algeria. In 1901, he founded the "Central Laboratory of Plant Pathology" and was its director until 1905. In 1907, he became the director of the newly organized "Bureau of Mycology and Phytopathology," which, in 1917, was reorganized into the Division of Mycology and Plant Pathology. Later this was elevated to a national "Institute," named for him, "Institut Jaczewski."

Professor Jaczewski was an unusually capable, productive worker, publishing much in the field of mycology and plant pathology. He was dominated to the last by worthy ideals of service through science, giving all he had in zealous pursuit of these, undaunted, undistraught, let happen what might. And withal, as his intimate associates and family knew him, ever the same courteous considerate gentleman; pure-minded, open-hearted, honest seeker of right and truth, interested in art, music, and all good things; "kind and amiable to everyone," "respected, admired, and loved by friends, associates, and pupils."

ROLAND THAXTER

AUGUST 28, 1858–APRIL 22, 1932

Roland Thaxter, in preparation for his life work, studied at Harvard University, receiving the A.B. degree Magna Cum Laude in 1882, devoted two years to work in the Harvard Medical School, and returned to graduate work under the Harris Fellowship to specialize in mycology and cryptogamic botany with Dr. Farlow, receiving the degrees of M.A. and Ph.D. in Natural History in 1888.

From 1888 to 1891 he served as Mycologist at the Connecticut Agricultural Experiment Station in New Haven, carrying on important pioneer work in the study and control of plant diseases, yet still continuing his activities in pure mycology. In 1891 he was called back to an assistant professorship as Dr. Farlow's associate, and in 1896 was given the full responsibilities of teaching and research in cryptogamic botany when Dr. Farlow relinquished all instruction save the guidance of occasional research students. In 1901 he became full professor, the position he occupied until 1919, when he became professor emeritus and honorary curator of the Farlow Library and Herbarium.

His publications comprise a list of 88 papers, chiefly concerned with fungi, but to some extent dealing with other groups of cryptogams and even with insects. The five volumes of his monograph of the Laboulbeniales stand out as one of the greatest of works in the history of mycology.

He exerted a distinct influence on plant pathology through his own brief but outstanding work in this field, through his inspiring example of the accuracy and completeness of his investigations, through the aid he gave to pathologists who consulted him in difficult mycological problems, and through the training and ideals he transmitted to students. In the course of his work he traveled extensively and made valuable and unusual collections. His eminence was internationally recognized by honorary memberships in learned societies and academies, by prizes, and by election to positions of honor in important societies. He was American editor for the Annals of Botany from 1907 until his death.

An outstanding scholar and investigator, an effective teacher, his nearly fifty years of devoted work exerted a significant influence far beyond his own sphere of endeavor, and his death was a great loss not only to mycology but to plant pathology as well.

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FACTORS AFFECTING THE SPORULATION OF PHYLLOSTICTA SOLITARIA IN ARTIFICIAL CULTURE

A. J. Mix

(Accepted for publication August 5, 1932)

Several investigators of the apple-blotch fungus, *Phyllosticta solitaria* E. and E., have experienced difficulty in obtaining sporulation in artificial culture. Clinton (3) reports that *P. solitaria* made rather slow growth in culture and "had not, after two months' development, given any sign of the formation of a spore stage." He does not state what medium he used. Scott and Rorer (13) found that pycnidium-like bodies were formed in abundance in all media, but that they usually were sterile. These investigators were able to secure spore-bearing pycnidia only on sterilized apple wood and on corn-meal agar. Lewis (10) obtained spores on apple wood but not on prune agar, potato agar, or potato cylinders. Roberts (12) found that on all ordinary solid culture media *P. solitaria* would form pycnidia but that they were sterile. Pycnidia with spores developed, however, after 2 or 3 months on sterilized apple twigs. Guba (6) had similar difficulty. He found that the fungus produced sterile pycnidia or "pycnosclerotia" on nearly all media employed, but sporulation occurred in his cultures only occasionally. He was unable to induce sporulation by the use of "roll cultures" or by exposing cultures to alternating high and low temperatures. His spore-bearing cultures failed to produce other spore-bearing cultures, and he concludes that there is no evidence of the occurrence of sporulating strains. More recently, Kohl (7) reports the formation of fertile pycnidia on corn-meal agar when the cultures contained the pieces of host tissue used in isolating the fungus. Subcultures from these sporulating cultures failed to produce fertile pycnidia.

Phyllosticta solitaria has been kept in pure culture in this laboratory for several years. One of the earliest observations on its behavior was that a large proportion of the cultures made on various common media produced spores. It seemed worth while, therefore, to study some of the factors that might influence sporulation of this fungus in culture, with the object of finding a medium on which sporulation would occur with some regularity.

INFLUENCE OF LOCALITY

Physiologically distinct strains of many fungi occur in different localities. It seemed possible that herein might lie the explanation of the fact

that *Phyllosticta solitaria* sporulated more readily in this laboratory than elsewhere. Accordingly, in 1925 a culture of the fungus, isolated from Tennessee material, was obtained from C. D. Sherbakoff. At the same time some blotch-cankered apple twigs were obtained from H. W. Anderson, and several isolations were made from them. The Tennessee and Illinois cultures produced spores as freely as did the Kansas cultures. Since as will appear below, there are sporulating and nonsporulating strains of the fungus in one locality, it seemed useless to pursue this point further.

INFLUENCE OF SOURCE OF CULTURE

It seemed possible that there might be some difference in behavior between cultures isolated from different parts of the host. Isolations were therefore made from twig cankers, fruits, and leaves; the fungus was grown on several media, chiefly potato agar without added sugar. The results are summarized below.

Cultures from twigs		Sporulating	73	Nonsporulating	44
"	" fruits	"	47	"	33
"	" leaves	"	31	"	37

There is clearly no significant difference as to ability to produce spores between cultures obtained from twigs, fruit, or leaves.

INFLUENCE OF TIME OF ISOLATION

Another possibility considered was that the time of year when isolations were made might influence the behavior of the fungus in culture. Guba (6) suggests this possibility. He obtained spores in culture from isolations made in February, and none from isolations made in autumn or early winter. His later attempts to obtain spores from isolations made in early spring were, however, unsuccessful.

There have been in this laboratory, during the past several years, nearly 300 cultures of *Phyllosticta solitaria,* each representing a separate isolation. These isolations were made at all seasons: from twig cankers at intervals throughout the whole year and from fruits and leaves during the spring and summer. The record kept on these cultures shows that several for every isolation-date have produced spores.

INFLUENCE OF LIGHT

Coons (4) found that light was necessary for formation of pycnidia by *Plenodomus fuscomaculans.* According to Coons and Levin (5), Levin (9), and Leonian (8), certain species of the Sphaeropsidales require light for pycnidium formation, while others do not. Guba (6), in experiments with *Phyllosticta solitaria,* found that the formation of sterile pycnidia (pycnosclerotia) was not inhibited by darkness.

The results shown in table 1 indicate that *Phyllosticta solitaria* belongs to the light-indifferent forms. In this experiment, as in those subsequently reported, duplicate cultures of each series were made in 125 cc. Erlenmeyer flasks, 40 cc. of the medium being placed in each flask. One series of cultures was exposed to the light from a window, the other was kept in a photographic dark room. The temperature of the 2 rooms was the same within 2 or 3 degrees throughout the experiment.

TABLE 1.—*Influence of light on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Pycnidia formed in light	Spores formed in light	Pycnidia formed in dark	Spores formed in dark
166	+	+	+	+
168	+	+	0	0
169	+	+	0	0
216	+	+	+	+
217	+	+	+	+
218	+	0	+	0
219	+	+	+	+
229	+	0	+	0
230	+	0	+	0
231	+	0	+	0

The performance of cultures 168 and 169 in this experiment might suggest that there are light-sensitive races of *Phyllosticta solitaria,* but whether any significance should be attached to the failure of these cultures to sporulate in the dark could be determined only by further experimentation.

SPORULATION ON COMMON MEDIA

The fungus has been found to produce spores on such common media as potato agar, oatmeal agar (Clinton's formula), corn-meal agar (Shear's formula), Difco corn-meal agar, green-apple agar, string-bean agar, bark-decoction agar, and potato-dextrose agar. The 2 last-named media, in suitable concentration, were found especially favorable to sporulation. They will be given further consideration below in connection with the discussion of quantity of food.

LIQUID VERSUS SOLID MEDIA

Liquid cultures are commonly used in nutrition studies with fungi, especially if quality of food is being considered. It appeared quite early in the present investigation that liquid culture media could not be used.

Cultures were made in various seed broths, potato-dextrose broth, and certain synthetic nutrient solutions. The fungus grew well, forming a considerable amount of submerged mycelium, but formation of pycnidia occurred only after the cultures had dried out enough to allow development of aerial mycelium, and pycnidia were not produced abundantly until the liquid was nearly or quite gone.

An attempt was made to use filter-paper pads in connection with liquid media as suggested by Leonian (8). They were, however, found unsatisfactory with this fungus. An experiment, for example, on quantity of food, in which parallel series of cultures were made using filter-paper pads wet with the nutrient solution and the same solution solidified with agar, resulted in very irregular sporulation on the pads. The agar cultures, on the other hand, gave regular and consistent results. Consequently, in the study of factors yet to be considered agar cultures were employed. There seems to be no objection to the use of agar in such experiments provided the fungus cannot use the agar itself as a source of carbon. Since *Phyllosticta solitaria* makes only negligible growth in cultures where agar supplies the only source of carbon, it can be assumed that agar is not utilized by this fungus.

INFLUENCE OF QUANTITY OF FOOD

The factor that, in these studies, has seemed to have the greatest effect on sporulation is quantity of food. Coons (4), who has studied the effect of food quantity on growth and reproduction of *Plenodomus fuscomaculans* (Sacc.) Coons, has shown that the range of food concentration within which growth of that fungus can take place is wider than that allowing formation of pycnidia and spores. Concentrations of food so low as to be near the subsistence limit did not permit sporulation in his cultures, while an overabundance of food caused excessive mycelial growth with attendant suppression of pycnidia. In his discussion Coons calls attention to the fact that, in most ordinary laboratory media, fungi are greatly overfed.

Phyllosticta solitaria, it will be seen, reacts readily to variations in food concentration. It is quite possible that the failure of previous investigators to obtain sporulation in culture was due to their using media of too high food content. Potato-dextrose agar, as it is ordinarily made in this laboratory (potatoes 140 gm., dextrose 10 gm., agar 20 gm., water 1,000 cc.), appears to be too rich for *P. solitaria*. It will allow sporulation but by no means so readily as more dilute media.

In a rich medium, such as potato-dextrose agar, growth of the fungus is thick and piled up, and separate pycnidia are not visible, but, by their coalescence they form a sclerotium-like crust. Some strains of the fungus form a whitish, aerial mycelium over this crust, others do not. In more

dilute media a radiate growth of appressed mycelium is formed, suggesting a common type of fruit blotch, and separate pycnidia appear throughout the colony. These are quite like the pycnidia formed in fruit blotches and twig cankers and are not like the sterile thick-wall pycnidia referred to by Guba (6) as pycnosclerotia. Photographs of cultures of *Phyllosticta solitaria,* showing types of colony formed in rich and in dilute media, are reproduced in figure 1.

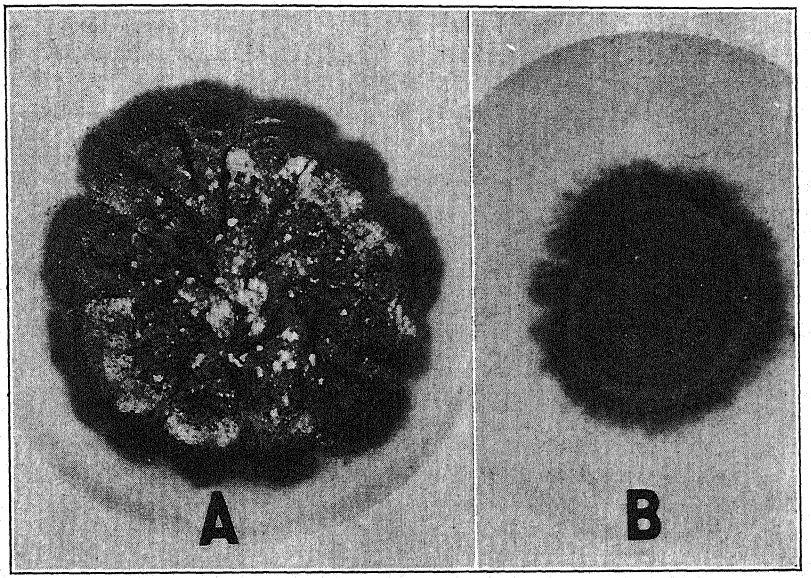


FIG. 1. Colonies of *Phyllosticta solitaria.* A. On S/1 potato-dextrose agar. B. On S/10 potato-dextrose agar. White dots in B are masses of pycnospores oozing from pycnidia.

The results of an experiment on quantity of food are shown in table 2. Potato-dextrose broth of the formula given above was taken as the standard and is designated in the table by the symbol S/1. This standard broth was diluted to $\frac{1}{2}$, $\frac{1}{4}$, etc., its original strength by means of distilled water. These dilutions are shown in the table by the symbols S/2, S/4, etc . All the broths were solidified with 2 per cent agar.

In recording the occurrence of pycnidia it was found convenient to use a system of notation like that of Coons (4), but with slightly different values. In this and the following tables the symbol 0 indicates the absence of pycnidia from a culture; + indicates the presence of from 1 to 25 pycnidia, ++ from 25 to 50; +++ from 50 to 100; ++++ 100 or more. As indicated earlier, all cultures were made in duplicate, each count, therefore, represents the average count of 2 cultures. Where, instead of separate

TABLE 2.—*Effect of different concentrations of potato-dextrose medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration	Pycnidia	Spores	Culture	Concentration	Pycnidia	Spores
Experiment 1							
168	S/1	Cr	+(f)	218	S/64	+++	+
	S/2	Cr	+		S/128	+	+
	S/4	++++	+		S/256	0	0
	S/8	++++	+	219	S/1	Cr	+(f)
	S/16	++++	+		S/2	Cr	+
	S/32	++++	0		S/4	Cr	+
	S/64	0	0		S/8	++++	+
	S/128	0	0		S/16	++++	+
	S/256	0	0		S/32	++++	+
216	S/1	Cr	+		S/64	+++	+
	S/2	Cr	+		S/128	0	0
	S/4	Cr	+		S/256	0	0
	S/8	++++	+	230	S/1	Cr	0
	S/16	++++	+		S/2	Cr	+
	S/32	++++	+		S/4	Cr	+
	S/64	+++	+		S/8	++++	+
	S/128	+	+		S/16	++++	+
	S/256	0	0		S/32	++++	+
217	S/1	Cr	+(f)		S/64	++	+
	S/2	Cr	0		S/128	0	0
	S/4	Cr	+		S/256	0	0
	S/8	++++	+	231	S/1	Cr	+
	S/16	++++	+		S/2	Cr	+
	S/32	++++	+		S/4	Cr	+
	S/64	+++	+		S/8	++++	+
	S/128	0	0		S/16	++++	+
	S/256	0	0		S/32	+++	+
218	S/1	Cr	+(f)		S/64	++	+
	S/2	Cr	+		S/128	0	0
	S/4	Cr	+		S/256	0	0
	S/8	++++	+				
	S/16	++++	+				
	S/32	++++	+				
Experiment 2							
166	8S	Cr	0	232	S/2	++++	+
	4S	Cr	0		S/4	++++	+
	2S	Cr	+(f)		S/8	++++	+
	S/1	Cr	+(f)		S/16	+++	+
	S/2	Cr	0	241	8S	Cr	0
	S/4	++++	+		4S	Cr	+
	S/8	++++	+		2S	Cr	+
	S/16	+	+		S/1	Cr	0
232	8S	Cr	+(f)		S/2	++++	+
	4S	Cr	+		S/4	++++	0
	2S	Cr	+(f)		S/8	++++	+
	S/1	Cr	+		S/16	++++	+

pycnidia, the sclerotial crust described above was formed, that fact is indicated in the tables by replacing the plus marks by the symbol Cr. Presence or absence of spores is indicated by the symbols + and 0, respectively.

In cases where only a few of the pycnidia in a culture bore spores (instead of nearly all the pycnidia, as was the usual case) this fact is indicated by the letter f in parentheses following the plus sign.

A second experiment in which the concentration of potato-dextrose broth was varied between limits of 8 times the usual strength and $\frac{1}{16}$ the usual strength is reported in table 2.

Examination of the results shown in table 2 indicates that the range of food concentration that will allow growth of *Phyllosticta solitaria* in culture is wider than the range for sporulation. As the food concentration is increased beyond that which allows only mycelial growth separate spore-bearing pycnidia occur in the cultures. With further increase in concentration these pycnidia become more numerous until their identity is lost and the attendant increase in mycelial growth results in the formation of the so-called "sclerotial crust." The pycnidia occurring in this crust may bear spores, but further increase in food concentration leads to reduction and even complete inhibition of spore formation.

It was impossible to estimate accurately the difference in amount of mycelial growth in these experiments. Mycelium in the more dilute media is very scanty, appressed, and often partly submerged. In the highest concentrations it is thick and piled up. The difference in volume of mycelium between the extremes is very large and a measurement of the radial spread of the colony would leave this difference out of account. In fact, the only consideration given to mycelial growth in these studies was that necessary to the final choice of a medium that would allow growth sufficiently vigorous to produce a large number of separate spore-bearing pycnidia. For example, it was decided after examination of the cultures in the 2 experiments just described that a convenient concentration of potato-dextrose broth that would allow sufficient growth, formation of separate, scattered pycnidia, and abundant sporulation would be that of $\frac{1}{10}$ the usual strength. Consequently, S/10 potato-dextrose agar has been used in further experimentation whenever a potato-dextrose medium was desired.

A similar effect of the concentration of food was found with apple-bark decoction agar. The bark decoction was prepared by boiling 50 gm. of bark from young apple twigs in a convenient quantity of water for 20 minutes, allowing the water to stand on the bark overnight, then, adding water to the filtered decoction to make a volume of 1,000 cc. The effect of bark decoction in standard strength and in various dilutions on sporulation is shown in table 3. Subsequent use of S/5 bark-decoction agar showed it to be a very favorable medium for the production of separate pycnidia and for sporulation.

An even narrower range of food concentration permitting sporulation was found with Richards' solution modified by substituting dextrose (1%) for the sucrose (5%) of that solution. This modified Richards' solution

TABLE 3.—*Effect of different concentrations of apple-bark decoction and of modified Richards' and Coons' media on pycnidium formation and sporulation of Phyllosticta solitaria*

Apple-bark decoction				Modified Richards' Medium			
Culture	Concentration	Pycnidia	Spores	Culture	Concentration	Pycnidia	Spores
166	S/1	++++	0	241	15S	Cr	0
	S/2	++++	+		10S	Cr	0
	S/4	+++	+		5S	Cr	0
	S/8	++	+		2S	Cr	0
	S/16	+	+		S/1	Cr	0
	S/32	0	0		S/5	++	+
	S/64	0	0		S/15	++	+
232	S/1	++++	0		S/50	0	0
	S/2	++++	+(f)		S/100	0	0
	S/4	++++	+	246	15S	Cr	0
	S/8	++	+		10S	Cr	0
	S/16	+	+		5S	Cr	0
	S/32	0	0		2S	Cr	0
	S/64	0	0		S/1	Cr	0
246	S/1	++++	0		S/5	0	0
	S/2	++++	+(f)		S/15	+	+
	S/4	++	+		S/50	0	0
	S/8	++	+		S/100	0	0
	S/16	0	0	297	15S	Cr	0
	S/32	0	0		10S	Cr	0
	S/64	0	0		5S	Cr	0
237	S/1	++++	0		2S	Cr	0
	S/2	++++	0		S/1	Cr	0
	S/4	++++	0		S/5	+++	+
	S/8	++++	+		S/15	+	+
	S/16	++	+		S/50	0	0
	S/32	0	0		S/100	0	0
	S/64	0	0	237	15S	Cr	0
234	S/1	++++	+(f)		10S	Cr	0
	S/2	++++	+		5S	Cr	0
	S/4	++++	+		2S	Cr	0
	S/8	++	+		S/1	Cr	0
	S/16	0	0		S/5	++	+
	S/32	0	0		S/15	+	+
	S/64	0	0		S/50	0	0
					S/100	0	0
				219	15S	Cr	0
					10S	Cr	0
					5S	Cr	0
					2S	Cr	0
					S/1	Cr	0
					S/5	0	0
					S/15	+	+
					S/50	0	0
					S/100	0	0

Modified Coons' Medium			
Culture	Dextrose	Pycnidia	Spores
	Per cent		
166	2	Cr	+
	1	Cr	+
	0.5	Cr	+
	0.2	++++	+
	0.1	++++	+
217	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++++	0
	0.1	++++	0
237	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++	0
	0.1	++++	0
247	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++++	+
	0.1	++++	+
342	2	Cr	0
	1	++++	0
	0.5	++++	+
	0.2	++++	+
	0.1	++	+

was used in standard strength and diluted. The results of this experiment also are shown in table 3. This did not seem to be a favorable medium for *Phyllosticta solitaria* in any strength and was not used further.

EXPERIMENTS INVOLVING BOTH QUALITY AND QUANTITY OF FOOD

The above series of experiments had shown that 2 media, S/10 potato-dextrose agar and S/5 bark-decoction agar, favored growth and sporulation of *Phyllosticta solitaria.* Since the composition of such media is unknown, and, at best, must be somewhat variable, it seemed desirable to devise a medium of known composition that could be relied on to induce formation of pycnidia and spores. It was decided to use as a basis for this experimentation a medium found by Coons (4) to be favorable for growth and sporulation of *Plenodomus fuscomaculans.* Since it was thought probable that dextrose would be a favorable source of carbon for *Phyllosticta solitaria,* as it is for fungi in general, and since it is cheaper than maltose, the former sugar was used.

Coons' medium, KNO_3 2 gm., KH_2PO_4 2.7 gm., $MgSO_4$ 1.25 gm., water 1,000 cc., was prepared with the following concentrations of dextrose: 2 per cent, 1 per cent, 0.5 per cent, 0.2 per cent, 0.1 per cent, and each solution was solidified with agar (Table 3). Two of the cultures (166 and 342) used in this experiment were known to sporulate consistently, 2 (217 and 237) were believed to be nonsporulators, and a fifth culture (247) was somewhat variable in performance. The same or a similar set of cultures was used in many of the following experiments.

It is evident from a study of table 3 that *Phyllosticta solitaria* reacts readily to quantity of carbon (supplied as dextrose) in the medium. The behavior of culture 166 is not fully shown in the table, since the quantity of spores produced (number of sporulating pycnidia) in 2 per cent dextrose was very small, and increased as the series descended. It was concluded that a suitable quantity of carbon for the needs of this fungus would be that furnished by a 0.10 per cent concentration of dextrose.

A second experiment in which the salt fraction of Coons' medium was reduced to $\frac{1}{10}$ standard strength and the dextrose concentration varied is reported in table 4. This, again shows the desirability of a low concentration of dextrose and indicates the further possibility that some or all of the salts might be used in lower concentration. Further experiments were, therefore, conducted to learn the most favorable concentrations of the various salts.

The results of an experiment in which the potassium nitrate content of the Coons' medium was varied and dextrose concentration kept at 0.10 per cent are shown in table 5. Sporulation was quite as good when the potassium nitrate was used in a concentration of 0.31 gm. per 1,000 cc. as

TABLE 4.—*Effect of different concentrations of dextrose in S/10 Coons' medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of dextrose	Pycnidia	Spores
	Per cent		
166	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	Cr	+
	0.1	+	0
217	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	Cr	0
	0.1	+	0
237	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++++	0
	0.1	+	0
247	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	Cr	0
	0.1	+	+
342	2	++++	0
	1	++++	+
	0.5	++++	+
	0.2	++++	+
	0.1	++++	+

when the medium contained 2.5 gm. per 1,000 cc. There is some indication that a concentration of 5 gm. of potassium nitrate per liter tends to suppress sporulation. Growth differences were not significant, somewhat the best growth being made in the cultures containing 1.25 gm. KNO_3 per 1,000 cc., but good growth occurred in the lowest concentration employed. In view of the general principle that lower food concentrations tend to favor sporulation, a concentration of 0.5 gm. KNO_3 per 1,000 cc. was chosen as convenient and probably favorable.

The next medium was made as follows: KNO_3 0.5 gm., $MgSO_4$ 1.25 gm., dextrose 1 gm., agar 20 gm., water 1,000 cc., and the concentration of KH_2PO_4 was varied. The results of this experiment are shown in table 6. Cultures 217 and 237, which seemed to be consistent nonsporulators, were replaced in this experiment by 2 others, 1 of which (299) was known to produce spores. Culture 166 was originally included, but the series became contaminated.

TABLE 5.—*Effect of different concentrations of KNO_3 in modified Coons' medium (containing 0.1% dextrose) on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of KNO_3 in gm. per liter	Pycnidia	Spores
166	5	++++	0
	2.5	++++	+
	1.25	++++	+
	0.63	++++	+
	0.31	++++	+
217	5	++++	0
	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
237	5	++++	0
	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
247	5	++++	0
	2.5	++++	+
	1.25	++++	0
	0.63	++++	+
	0.31	++++	+
342	5	++++	+
	2.5	++++	+
	1.25	++++	0
	0.63	++++	+
	0.31	++++	+

It appears from a study of this table that concentration of KH_2PO_4 is not so important as that of KNO_3 or of dextrose. It can be varied within rather wide limits without greatly affecting the behavior of the fungus. Since a concentration of 1.25 gm. KH_2PO_4 in 1,000 cc. of medium allowed good growth and sporulation, this concentration was chosen for the new medium. It seemed desirable to keep the concentration of KH_2PO_4 above that of the other salt constituents for two reasons: the demand of fungi for phosphorus is known to be comparatively high and the use of acid phosphate tends to produce an acid reaction in the medium.

It remained to determine the proper amount of $MgSO_4$ for this new medium. The results of an experiment designed to discover this are shown in table 7. The fungus did not seem to react markedly to changes in $MgSO_4$ concentration within the limits of the experiment. In keeping with the plan

TABLE 6.—*Effect of different concentrations of KH_2PO_4 in modified Coons' medium (containing 0.05% KNO_3, 0.1% dextrose) on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of KH_2PO_4 in gm. per liter	Pycnidia	Spores
166	5	++++	0
	(Remainder of series contaminated)		
231	5	++++	0
	2.5	++++	0
	1.25	++	0
	0.63	+	0
	0.31	+	0
247	5	++++	+(f)
	2.5	++++	+
	1.25	++++	0
	0.63	++++	+
	0.31	++++	0
299	5	++++	+
	2.5	++++	+
	1.25	++++	+
	0.63	+++	+
	0.31	+++	+
342	5	++++	+(f)
	2.5	++++	+(f)
	1.25	++++	+
	0.63	++++	+
	0.31	++++	+

to have the total food concentration low, a concentration of 0.75 gm. $MgSO_4$ was chosen as convenient.

The new medium, hereafter referred to as the K.U. medium, is constituted as follows: KNO_3 0.5 gm., KH_2PO_4 1.25 gm., $MgSO_4$ 0.75 gm., dextrose 1 gm., water 1,000 cc. When 2 per cent agar is added this medium allows good growth and sporulation. Its acidity is pH 5.2 to pH 5.4 This, as will be shown later, is a favorable degree of acidity for the fungus in question.

It was, of course, not known whether any one salt was present in this medium in exactly the best amount nor whether the physiological balance between the various constituents was ideally realized. A series of cultures was, therefore, made according to the triangle system well known in plant physiology. The concentration of each salt was varied between limits of 0.25 gm. and 2.00 gm. per 1,000 cc. with intervals of 0.25 gm. between adjacent cultures. Each culture was made in duplicate and 5 sporulating strains of *Phyllosticta solitaria* were used. The results of this experiment

TABLE 7.—*Effect of different concentrations of $MgSO_4$ in modified Coons' medium (containing 0.05% KNO_3, 0.125% KH_2PO_4, 0.1% dextrose) on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of $MgSO_4$ in gm. per liter	Pycnidia	Spores
166	2.5	++++	+
	1.25	++++	+(f)
	0.63	++++	+(f)
	0.31	+	+
231	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
247	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
299	2.5	Cr	+
	1.25	++++	+
	0.63	++++	+
	0.31	++++	0
342	2.5	++++	+
	1.25	++++	+
	0.63	++++	+
	0.31	++++	+

showed that best sporulation occurred when the concentrations of the various salts per 1,000 cc. were as follows:

A.	KNO_3	0.50	gm.	KH_2PO_4	1.25	gm.	$MgSO_4$	0.75	gm.
B.	"	0.25	"	"	1.25	"	"	1.00	"
C.	"	0.25	"	"	1.00	"	"	1.25	"
D.	"	0.25	"	"	0.75	"	"	1.50	"
E.	"	0.50	"	"	0.50	"	"	1.50	"

This would indicate that a satisfactory medium for the purpose desired might contain: KNO_3 0.25 to 0.50 gm., KH_2PO_4 0.50 to 1.25 gm., $MgSO_4$ 0.75 to 1.50 gm., water 1,000 cc.

SOURCES OF NITROGEN FOR PHYLLOSTICTA SOLITARIA

Although the K.U. medium described above is entirely satisfactory for growth and sporulation of *Phyllosticta solitaria,* it seemed advisable to investigate other possible sources of nitrogen and carbon.

The first experiments were designed to test the availability of asparagin as a nitrogen source as opposed to potassium nitrate. For this purpose a

medium devised by Brown (2) and intended to approximate the constitution of potato-dextrose agar was used. The salt constituents of this medium are as follows: asparagin 2.0 gm., K_3PO_4 1.25 gm., $MgSO_4$ 0.75 gm. per 1,000 cc. In 1 experiment this medium was used as just given and the dextrose concentration was varied as follows: 2 per cent, 1 per cent, 0.5 per cent, 0.2 per cent, 0.1 per cent. In this series of cultures growth was slow and restricted in amount and, except in the lowest concentration of dextrose, consisted in a thick, piled-up sclerotial crust, such as had been found in previous experiments when excess food was present. Sporulation occurred in all cultures but separate pycnidia formed only in the cultures containing 0.1 per cent dextrose.

TABLE 8.—*Effect of different concentrations of asparagin in Brown's medium (containing 0.1% dextrose) on growth and sporulation of Phyllosticta solitaria*

Culture	Concentration of asparagin in gm. per liter	Pycnidia	Spores
231	2.0	0	0
	1.0	0	0
	0.5	0	0
	0.2	+	0
247	2.0	Cr	0
	1.0	Cr	0
	0.5	+	0
	0.2	+	0
299	2.0	Cr	0
	1.0	Cr	0
	0.5	++++	+
	0.2	++++	+
342	2.0	0	0
	1.0	0	0
	0.5	0	0
	0.2	++++	+

In a second experiment (Table 8) the dextrose concentration was kept at 0.1 per cent and the concentration of asparagin was varied. The suppression of sporulation by excessive concentrations of asparagin is evident. But, even in the lowest concentrations, mycelial growth was restricted. It was concluded that no modification of Brown's medium would be particularly suitable for *Phyllosticta solitaria.*

In a further experiment (table 9) potassium nitrate of Coons' medium was replaced by other nitrogen compounds. The amount of each compound used was such as to supply as much nitrogen (0.28 gm. per liter) as that contained in the 2 gm. per liter of potassium nitrate in the original Coons'

TABLE 9.—*Effect of different sources of nitrogen in modified Coons' medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Source of N	Pycnidia	Spores
166	KNO_3	++++	+
	NH_4NO_3	++++	0
	$(NH_4)_2SO_4$	++++	+
	NH_4Cl	++++	+(f)
	Ammonium acetate	0	0
	" oxalate	No growth	No growth
	Asparagin	++++	0
	Albumen	++++	+
	Peptone	++++	0
231	KNO_3	++++	+
	NH_4NO_3	++++	0
	$(NH_4)_2SO_4$	++++	0
	NH_4Cl	++++	0
	Ammonium acetate	++++	0
	" oxalate	No growth	No growth
	Asparagin	++++	0
	Albumen	++++	0
	Peptone	++++	0
247	KNO_3	++++	+
	NH_4NO_3	0	0
	$(NH_4)_2SO_4$	0	0
	NH_4Cl	0	0
	Ammonium acetate	++++	0
	" oxalate	No growth	No growth
	Asparagin	0	0
	Albumen	++++	0
	Peptone	++++	0
299	KNO_3	++++	+
	NH_4NO_3	++++	0
	$(NH_4)_2SO_4$	++++	0
	NH_4Cl	++++	0
	Ammonium acetate	Cr	+
	" oxalate	No growth	No growth
	Asparagin	++++	+
	Albumen	++++	+
	Peptone	++++	+
342	KNO_3	++++	+
	NH_4NO_3	++++	+(f)
	$(NH_4)_2SO_4$	++++	+
	NH_4Cl	++++	+
	Ammonium acetate	Cr	0
	" oxalate	No growth	No growth
	Asparagin	Cr	0
	Albumen	++++	+
	Peptone	Cr	0

medium. Of the various compounds tried potassium nitrate seemed to be most favorable to sporulation, with albumen next. Asparagin and peptone seemed somewhat less favorable and the other compounds distinctly unfavorable. (In the case of compounds containing both nitrogen and carbon, parallel cultures without dextrose showed that none of these compounds furnished much available carbon for this fungus).

SOURCES OF CARBON FOR PHYLLOSTICTA SOLITARIA

The K.U. medium described above contains dextrose as a source of carbon. Coons (4) found maltose especially favorable for growth and pycnidium formation of *Plenodomus fuscomaculans*. It seemed desirable to compare these and other carbon compounds as to availability for *Phyllosticta solitaria*.

In order to avoid concentration differences Coons used equimolecular solutions of various carbohydrates. It seems that carbon-equivalent solu-

TABLE 10.—*Effect of different sources of carbon in K. U. medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Source of C	Pycnidia	Spores	Culture	Source of C	Pycnidia	Spores
166	Arabinose	++++	+		Maltose	+	0
	Xylose	Cr	0		Lactose	+++	+
	Rhamnose	Cr	0		Raffinose	++	0
	Dextrose	+	0		Starch	0	0
	Mannose	++++	+(f)		Dextrin	0	0
	Galactose	++++	+	299	Arabinose	++++	0
	Levulose	+	0		Xylose	.....	.....
	Sucrose	++++	+		Rhamnose	+	0
	Maltose	++++	0		Dextrose	++++	+
	Lactose	++	0		Mannose	++++	+
	Raffinose	++	0		Galactose	++	0
	Starch	++	0		Levulose	++++	+
	Dextrin	+	0		Sucrose	++++	+
231	Arabinose	++++	0		Maltose	++++	+
	Xylose	.....	.....		Lactose	++++	+
	Rhamnose	+	0		Raffinose	++++	+
	Dextrose	++++	0		Starch	++++	+
	Mannose	++++	0		Dextrin	+++	+
	Galactose	++	0	342	Arabinose	+	0
	Levulose	++	0		Xylose	.....	.....
	Sucrose	++++	0		Rhamnose	+	+
	Maltose	++++	0		Dextrose	++++	+
	Lactose	++++	0		Mannose	++++	+
	Raffinose	++++	0		Galactose	++++	+
	Starch	++++	0		Levulose	+++	+
	Dextrin	++++	0		Sucrose	+	+
247	Arabinose	Cr	+(f)		Maltose	+	0
	Xylose	.....	.....		Lactose	+	0
	Rhamnose	0	0		Raffinose	+	+
	Dextrose	++++	+		Starch	+	0
	Mannose	++++	+		Dextrin	+	0
	Galactose	+	+				
	Levulose	++++	+				
	Sucrose	++++	+				

tions would be preferable. Since carbohydrates furnish carbon to the fungus, 2 solutions, to be comparable on the basis of food value, should contain equal weights of carbon per unit volume; and, in view of the well-known tolerance by fungi of differences in osmotic concentrations, the molecular concentration should, in fairly dilute solutions, be of secondary importance. For example: in a molecular solution of dextrose there are 72 gm. of carbon per liter; in a molecular solution of sucrose there are 144. In a 1 per cent solution of dextrose there are 40 gm. of carbon per liter, and in a 1 per cent solution of sucrose there are 42. To be strictly comparable, if 1 solution contains 1 per cent dextrose, the other solution should contain 0.95 per cent sucrose. Since the standard for comparison in these studies was a solution containing 0.1 per cent dextrose, sucrose and similar carbon compounds should have been used in a concentration of 0.095 per cent. Practically, the difference between 0.1 per cent and 0.095 per cent solutions would seem to be negligible. The procedure, therefore, was to add the different carbohydrates used in concentrations of 1 gm. per liter to the culture medium: KNO_3 0.5 gm., KH_2PO_4 1.25 gm., $MgSO_4$ 0.75 gm., agar 20 gm., water 1,000 cc.

It is apparent (table 10) that a number of sugars will furnish available carbon for growth and sporulation of *Phyllosticta solitaria*, a notable exception being xylose. There seems to be little choice between dextrose, levulose, mannose, and sucrose. Mycelial growth was somewhat more vigorous on sucrose than on any of the others. Maltose does not seem to be a good sugar for this fungus. Not only did it inhibit sporulation, but it gave rise to a restricted growth of appressed and buried mycelium. Many of the pycnidia also were buried in the medium.

INFLUENCE OF HYDROGEN-ION CONCENTRATION

A series of cultures was prepared in which the initial hydrogen-ion concentration of the K.U. medium was varied by the addition of suitable amounts of lactic acid or of sodium hydroxide. This series ranged from pH 3.6 to pH 7.2, with intervals between each culture of 0.2 pH. Strains 342 and 299 (good sporulators) were used and the whole series was run in duplicate. It was found that abundant formation of pycnidia and good sporulation occurred in all cultures from pH 4.2 to pH 5.8, inclusive. The remainder of the series, from pH 6.0 to pH 7.2, showed in some cases the formation of a sclerotial crust but in no case formation of pycnidia or spores. Mycelial growth of strain 299 was best at pH 5.4, of 342 at pH 5.2. It is to be noted that this is the approximate hydrogen-ion concentration of the normal K.U. medium.

INFLUENCE OF TEMPERATURE

Guba (6) has studied the effect of temperature on the time of appearance of growth and the time necessary for pycnidium (pycnosclerotium)

TABLE 11.—*Effect of temperature on growth, pycnidium formation, and sporulation of Phyllosticta solitaria*

First Experiment—Observations at end of 30 days					Second Experiment—Observations at end of 20 days				
Culture	Temperature	Growth	Pycnidia	Spores	Culture	Temperature	Growth	Pycnidia	Spores
	° C.	Mm.				° C.	Mm.		
166	12–14	2	0	0	231	6–8	0	0	0
	17–19	10	+	0		13–15	0	0	0
	22–24	20	++	0		18–20	20	0	0
	26–28	25	++++	0		23–24	20	+++	0
	31–32	5	+	0		27–29	25	0	0
	36–37	0	0	0		31–33	20	0	0
231	12–14	5	0	0	247	6–8	0	0	0
	17–19	20	++++	0		13–15	10	Cr	0
	22–24	30	+++	0		18–20	20	0	0
	26–28	45	++++	0		23–24	30	0	0
	31–32	40	+	0		27–29	25	0	0
	36–37	0	0	0		31–33	20	++	+
247	12–14	5	0	0	299	6–8	0	0	0
	17–19	15	Cr	0		13–15	8	Cr	0
	22–24	40	+++	+		18–20	15	++++	+
	26–28	45	++++	+		23–24	25	++	+
	31–32	35	++++	0		27–29	35	++++	+
	36–37	0	0	0		31–33	10	0	0
299	12–14	5	0	0	342	6–8	0	0	0
	17–19	20	Cr	0		13–15	5	0	0
	22–24	35	++++	+		18–20	20	+++	0
	26–28	40	++++	+		23–24	25	+++	+
	31–32	20	+++	0		27–29	30	++++	+
	36–37	0	0	0		31–33	10	0	0
342	12–14	5	0	0					
	17–19	15	+++	0					
	22–24	20	++++	+					
	26–28	35	++++	+					
	31–32	10	+	0					
	36–37	0	0	0					

formation by *Phyllosticta solitaria*. He states that the minimum temperature for growth lies between 5° and 10° C., the optimum between 25° and 30° C., and the maximum between 30° and 35° C. Pycnidia were formed at all temperatures at which growth occurred.

In the experiments reported in table 11 the cultures were placed in a battery of temperature chambers modelled after those described by Livingston and Fawcett (11), the cooling being accomplished by means of an electric refrigeration unit.

The results of these experiments indicate that the minimum temperature for growth lies between 8° and 12° C. (Guba found that growth began after 25 to 35 days in cultures held at 10° C.). The maximum temperature for growth seems to lie between 33° and 35° C., and the optimum between 26° and 29° C. The temperature range for sporulation is narrower than that permitting growth. One culture produced spores at 33° C., and 1 at 18° C., but good sporulation of all cultures occurred only between 22° and 29° C. Although not clearly indicated, the optimum temperature for sporulation perhaps coincides with the optimum for growth.

Phyllosticta solitaria seems to have rather high temperature requirements for sporulation in culture. One is tempted to seek in this peculiarity of the fungus an explanation of the geographic distribution of apple blotch. This disease is less destructive in the northern part of its range and is absent from the northern apple-growing regions. It was reported by Thomas (15) as present in New York in 1922, but did not become established. Judging from the temperature experiments reported here, a consecutive period of 480 hours (20 days) at approximately 70° F. will allow sporulation in culture. It would not be safe, however, to argue from this as to the minimum time and temperature required for sporulation in nature. Aside from the fact that the fungus in nature is growing on a different substratum, there is the possibility that the apple twig may have at times a temperature somewhat above that of the surrounding air, as shown by Stevens (14) for twigs of certain plants. Perhaps a study of the temperature requirements of this fungus for spore germination and infection may help to explain the failure of apple blotch to become established northward. It is well, however, to recall in this connection the opinion expressed by Anderson (1), who, from observation of the northward advance of apple blotch in Illinois, feels that it is only a matter of time until the disease becomes serious in northern localities.

OCCURRENCE OF SPORULATING STRAINS

In the early part of this work, isolations were made from various sources and at various times of the year, and the fungus was grown on various media, principally potato agar (without sugar) and potato-dextrose agar. Of these early cultures 141 produced spores at some time or other; 114 did

not. During the latter part of the work 30 cultures were specially tested as to sporulation on S/10 potato-dextrose agar, S/5 bark-decoction agar, Difco corn-meal agar, and K.U. agar. Twenty cultures produced spores on one or more of these media; 10 did not. In several of the experiments reported above there were included sporulating cultures, nonsporulating cultures, and weakly sporulating cultures. It is apparent from a reference to the tables that none of these cultures changed its character under the influence of any of the factors studied. It is quite evident that there are sporulating and nonsporulating strains of *Phyllosticta solitaria*. This fact may possibly account for the failure of other investigators to obtain sporulation in culture, though it is evident that in many cases these investigators were employing media too rich for good sporulation.

Presumably both nonsporulating and sporulating strains might be secured from one parent-sporulating strain, but the only attempt made to do this was unsuccessful. A number of monospore isolations were secured from pycnidia produced in cultures 168, 247, 299, and 342, and the subcultures were tested on K.U. agar. They all produced spores.

SALTATION IN CULTURE

The appearance of light colored sectors devoid of pycnidia in the dark pycnidium-bearing cultures of *Phyllosticta solitaria* has been observed several times during the course of this work. Such sectors have usually arisen in cultures low in food materials or when the nutrients used have not been favorable to the development of the fungus. Transfers from such sectors have yielded light colored, nonsporulating cultures distinct from the parent type. Some of these saltants have preserved their character through several successive transfers to fresh media; others have reverted to the parent type after 1 or 2 transfers. This indicates that non-sporulating strains may arise from sporulating strains by saltation. Occasional loss of sporulating capacity has been observed in stock cultures kept in the laboratory over a period of years. It is thought that this may have come about through the accidental transfer of a nonsporulating saltant. On the other hand, many strains of the fungus have retained their capacity to sporulate after having been kept alive by mycelial transfer more than 6 years.

AUTOLYSIS

It has been observed that there is a favorable period for finding of spores in culture. Pycnidia with spores may generally be found in a culture 10 days or 2 weeks old. Some time later autolysis sets in. Spores seem to be digested first, mycelium later and more gradually. Spores can never be found in very old cultures. The onset of autolysis is, of course, variable and is influenced by such factors as medium and temperature. In general, cultures 2 months old or older may not be expected to show spores.

VIABILITY OF SPORES FROM CULTURE

A number of tests have shown the spores produced in culture to be viable. Spores from culture have been used for inoculating young, potted apple trees growing in the greenhouse. Such inoculations have resulted in production of typical apple-blotch leaf spots.

DISCUSSION

It will be apparent from the results of this study that the procedure for obtaining cultures of *Phyllosticta solitaria* yielding an abundant supply of spores is simple. It consists, first, in making a considerable number of isolations to insure that some of the strains secured will be capable of sporulation, next, in growing the fungus on a suitably dilute medium, such as S/10 potato-dextrose agar, S/5 bark-decoction agar, or the K.U. agar described above.

SUMMARY

The apple-blotch fungus, *Phyllosticta solitaria* has been found to produce pycnidia and spores on a variety of media.

Spore production in culture is not influenced by the locality from which the fungus is obtained, by the part of the host from which the fungus is isolated, nor by the time of year when isolations are made.

The fungus sporulates equally well whether the cultures are kept in darkness or in the light.

Solid media are more favorable to spore production than liquid media.

Quantity of nutrient apparently exercises the greatest influence on spore production in culture. A too rich medium may inhibit sporulation; one that is too lean may not support enough growth to allow sporulation. Potato-dextrose agar of $\frac{1}{10}$ the usual strength and bark-decoction agar of $\frac{1}{5}$ the usual strength have been found favorable to the production of separate fertile pycnidia.

By varying the concentration of the various constituents of Coons' medium a medium favorable to sporulation has been obtained. This medium contains: KNO_3 0.5 gm., KH_2PO_4 1.25 gm., $MgSO_4$ 0.75 gm., dextrose 0.10 gm., agar 20 gm., water 1,000 cc. Its reaction is pH 5.2 to pH 5.4.

The concentrations of the salt constituents are not important within certain limits, and a favorable medium might contain: KNO_3 0.25 to 0.50 gm., KH_2PO_4 0.50 to 1.25 gm., and $MgSO_4$ 0.75 to 1.50 gm., per 1,000 cc.

Of several compounds tested as sources of nitrogen for *P. solitaria,* potassium nitrate proved most favorable to growth and sporulation, albumen seemed next in value, with asparagin and peptone less favorable, and other compounds unfavorable.

As sources of carbon, dextrose, levulose, mannose, and sucrose were found equally valuable. Maltose was found unfavorable to growth and sporulation, and xylose did not support growth.

On the synthetic medium described above the fungus was found to produce fertile pycnidia when the initial hydrogen-ion concentration was between pH 4.2 and pH 5.8.

The following are the cardinal temperatures for growth of *P. solitaria* in culture: minimum between 8° C. and 12° C., optimum between 26° C., and 29° C., maximum between 33° C. and 35° C. The temperature range favorable to sporulation lies between 22° C. and 29° C. It does not seem possible to find in these temperature relations of the fungus any explanation of its present distribution in nature.

When many isolations of *P. solitaria* are made both sporulating and nonsporulating strains are found. It has not been possible to induce sporulation in nonsporulating strains.

Nonsporulating strains arise from sporulating strains by saltation.

Autolysis of mycelium and spores occurs in old cultures of *P. solitaria*.

Spores from culture were found viable and were successfully used in inoculation experiments.

DEPARTMENT OF BOTANY,
UNIVERSITY OF KANSAS.

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GROWTH RELATIONS IN CULTURE OF THE COTTON-ROOT-ROT ORGANISM, PHYMATOTRICHUM OMNIVORUM

ELIZABETH JANE MOORE

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INTRODUCTION

Since infection is an expression of the equilibrium between host and parasite, the selection of resistant varieties must often depend on minute change of this balance. Quantitative inspection of this balance may show new factors or factor intensities that may be selected for building up varietal resistance.

Since no varieties of cotton resistant to root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, have been recognized, this study has been undertaken in an effort to determine the effect of resistance on the growth of the parasite and to record any variability of this effect in the cotton plant.

HISTORICAL

Monocotyledonous plants are considered immune from the attack of Phymatotrichum root rot, although King and Loomis (9), in 1929, reported active growth of the fungus on the tissue of monocots. In 1931, Ezekiel, Taubenhaus, and Fudge (4) reported an investigation of the nutritional requirements of the fungus. In 1932 the same workers (5) reported studies on the growth of this fungus in various plant juices and correlated this growth with the susceptibility and resistance of the plants to the disease. Growth was measured by the dry weight of the hyphae developed in the juices, undiluted and diluted with distilled water or nutrient solution. In nondiluted autoclaved juices of resistant plants the growth was greatly inhibited, while growth in the juices of susceptible plants was abundant. On dilution of the juices of resistant species a good growth was secured. Monocotyledonous plants evidently contain some substances that, when present in sufficiently large concentrations, inhibit the growth of the root-rot fungus. The resistance of the plants is at least partly based on the presence of these materials.

The assumption may safely be made that growth activity varies with the vigor of the fungus and, as a consequence, offers a valuable means of determining the effects of environmental conditions on host infection and subsequent fungus development. Numerous studies have shown that growth in fungus hyphae is apical. Reinhardt, according to Jost (2, pp. 5–6), in 1856, showed that growth takes place largely at the tip of the apical cell, decreasing basally. The studies of Brown (3) showed typical sigmoid

curves for mycelial growth, with an initial period of slow growth, a period in which maximum growth was obtained, and a constant period, declining with the accumulation of metabolic waste products.

Smith (11 and 12) reported detailed studies on the growth of individual hyphae. By following the development of individual hyphae on inverted miniature plates, he showed that only apical growth occurred on the mycelia of *Botrytis cinerea* Pers.; that cells once separated from the apex showed no elongation. In each hypha the rate at which the tip advanced was not constant. In short, hyphae growth was very slow and increased with elongation up to 20 to 30 fold and finally decreased. This increase was considered to result from the increasing septal resistance to the transport of nutrients through the hypha and ultimately to result in branching. Branches show a sequence of growth rates similar to that of the parent hypha and reduce the rate of elongation of the latter. If the curve of the hyphal length was plotted against the time, it was concave to the time axis; but, if the entire hyphal system was considered, a straight line resulted in most cases.

Fawcett (6) showed that fungi exhibit no tendency toward senility if the staling of the media is prevented and that the rate of growth of the entire hyphal system remains constant for considerable periods. Haines (7 and 8), working with *Sporotrichum carnis* Brooks & Hansford, obtained the logarithmic curve typical of hyphal growth and pointed out that linear measurements are true representations of volume growth since, during the logarithmic period, the diameter is constant. With saprophytic Actinomyces the logarithmic curve of growth rates was also typical, the logarithms of the length plotted against the time giving a straight line.

METHOD

Cultures of strains 24 and 39 were secured from J. J. Taubenhaus and cultures grown from sclerotia obtained from the Temple and San Antonio, Texas, experiment stations. The stock cultures were maintained on potato-dextrose agar at 27 to 29° C.

For observation a modified hanging-plate method was followed. Paraffin cells, 2 in. × 3 in., were attached to large microscope slides enclosed by a large cover slip, carrying the agar plate.

A ruled cover slip was sterilized in a Petri dish, and a thin agar plate poured by introducing 1 cc. of the medium by means of a pipette. A wax-pencil line on the cover slip prevented the medium from flowing to the edge. A 4 mm. cube from the peripheral zone of an actively growing culture was used as inoculum. After 12 to 18 hours the inoculum was removed in order to reduce the complexity of the hyphal system. The plate was then inverted over the cell, which had been sterilized by $HgCl_2$ solution and rinsed

in sterile water, and sealed by running a hot spatula around the edge of the cell. On such a plate the fungus will remain in an actively growing condition for weeks. The cells were incubated at 27 to 29° C. and received no illumination except at the time of observation. The mycelia were observed and measured without disturbing the cell.

Measurements of the hyphae were made by means of a Filar micrometer ocular at 2 to 3 hour intervals over varying periods. Both the parent hyphae and the entire hyphal system were recorded, the first measurement being taken as zero. Since elongation is purely apical, measurements were facilitated by use of the reference lines on the cover slips.

A potato-dextrose medium (250 gm. potato steamed 1 hr. in 500 cc. distilled water, 1 per cent dextrose and 2 per cent agar) was used as a stock and also as a standard for growth to compare with that on other media. Decoction media were prepared from various roots by steeping 1 gm. dry ground root in 200 cc. water for 1 hour, filtering, and adding 2 per cent agar. Root decoction was also added to potato dextrose in the same (1:200) ratio. All media were autoclaved at 15 lb. additional pressure for 20 minutes and remelted when desired in an Arnold steamer.

EXPERIMENTAL RESULTS

First experiments were made to establish a standard rate of growth of individual hyphae of Phymatotrichum under given cultural conditions. Potato-dextrose medium was chosen, since it is a favorable one for this fungus. Growth rates were established for strains 24 and 39 and for a culture obtained from a sclerotium. The average growth, calculated from measurements made on 2 to 14 hyphae in each of these experiments, is recorded in table 1 in the columns following the experiment numbers. The averages of all observations made on a given strain are shown in the final horizontal line of each division. These total averages are summarized in figure 1.

It will be seen that strains 24 and 39 maintained a very similar rate of elongation and that the sclerotium culture exhibited a growth rate of a much higher value. When it is remembered that these observations began 12 to 18 hours after the inoculation of the plates and that growth was well under way, the short period of observation is thought to represent the growth rates under favorable cultural conditions. There appears to be little difference in the growth rates of strains 24 and 39; this can be seen not only in figure 1 but also from the final column of averages in table 1, in which both strains show considerable variability and a total average of 0.158 and 0.146 mm. per hour, respectively. Since these averages include readings over a longer period than have been shown in figure 1, no significant differences in the growth rates of these strains appeared during the periods

TABLE 1.—*Growth of individual hyphae and hyphal systems*[a] *of Phymatotrichum omnivorum as grown on potato-dextrose agar. (Averaged from 2 to 14 hyphae. Exception Experiment XIV, 1 hypha measured)*

Experiment	Strain	Average total growth in mm.									Average rate
		Hrs. 6	12	18	24	30	36	42	48	54	
I	24	0.603	1.215	1.818	2.34	3.663	4.95	6.426	7.47	11.88	0.22
II	"	1.08	1.935	2.835	3.726						0.155
III	"	0.486	1.179	2.034	2.448	2.817	3.915	4.41	4.995	5.04	0.090
V	"	1.17	2.646	4.176	5.949						0.247
XV	"	0.405	1.485	2.475	3.402						0.141
XXI	"	0.36[a]	1.54[a]								0.128
XXII	"	0.765[a]	1.35[a]	2.250[a]							0.125
Average	"	0.695	1.621	2.598	3.573	3.24	4.432	5.418	6.23	8.46	0.158
V	39	0.612	1.242	2.331	3.447						0.143
IX	"	0.864	1.899	2.862	3.825	4.608	4.86				0.135
XIV	"	0.918[a]	1.638[a]	2.043[a]	2.448[a]						0.102
XV	"	0.945[a]	1.395[a]	1.575[a]							0.087
XXI	"	0.918[a]	2.988[a]								0.249
XXII	"	0.720[a]	0.990[a]	2.925[a]							0.162
Average	"	0.829	1.629	2.347	3.240						0.146
XX	Sclerotial	2.34[a]	3.627[a]								0.302
XXI	"	3.24[a]	6.714[a]								0.559
XXII	"	2.745[a]	3.905[a]	8.100[a]							0.450
Average	"	2.775	4.748	8.100							0.437

[a] Denotes measurement of entire hyphal system.

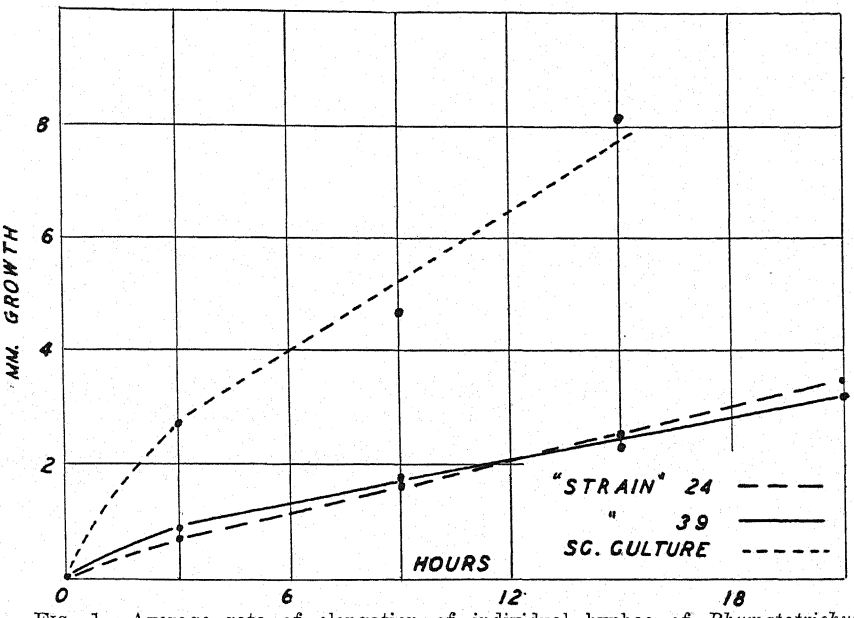


FIG. 1. Average rate of elongation of individual hyphae of *Phymatotrichum omnivorum* on potato-dextrose agar.

of observation. In contrast to this, the sclerotial culture shows an average nearly 3 times that of strains 24 and 39. This culture may be regarded as more vigorous than 24 and 39, although it is more probable that prolonged culture on artificial media of the two strains has resulted in a considerable decrease in vigor.

In order to test the effects of decoctions of dried roots on the growth of Phymatotrichum, a preliminary study was made, using potato dextrose in comparison with a decoction of dried ground cotton root plus 2 per cent agar. Two concentrations of decoction were made, 1:100 and 1:500. Average rates per hour shown by strain 24 were potato dextrose, 0.161; cotton-root decoction 1:100, 0.283; and 1:500 decoction, no growth. For further study a concentration of 1:200 or less was used. The averages obtained from 2 to 12 hyphae are given in table 2. The decoctions were of cotton and corn roots, the former plant being highly susceptible and the latter resistant to this disease in the field (13, 14).

It will be seen that strain 24 in experiments XI and XII showed an average growth rate of 0.099 and 0.157, giving a general average of 0.128. A similar average for a somewhat longer period gave a growth rate of 0.077 on the corn-root-decoction medium. If these rates are compared with the rate on potato dextrose (0.158), it will be seen that neither decoction medium was so favorable for growth and that the growth rate in corn-root-

TABLE 2.—*Growth of individual hyphae of Phymatotrichum omnivorum in root decoctions. (Averaged from 2 to 12 hyphae)*

Experiment	Decoction	Strain	Average total growth in mm. Hrs. 6	12	18	24	30	36	42	48	54	60	66	Average rate
XI	Cotton 1: 200	24	0.658	1.152	1.674	2.61	2.97							0.099
XII	" "	24	1.206	2.385	4.698	3.402	4.132	4.599	6.156	7.596				0.157
	Average		0.932	1.768	3.186	3.006	3.551	4.599	6.156	7.596				0.128
XI	Cotton 1: 200	39	1.449	2.340	3.150	3.340								0.101
XII	" "	39	1.269	2.700	4.356	6.012	7.497	8.550	11.70	13.59				0.283
	Average		1.359	2.520	3.753	4.976	7.497	8.550	11.70	13.59				0.192
X	Corn 1: 250	24	0.869	2.151	3.114	3.213	3.652	4.266	4.266	4.473	4.68	4.89	5.10	0.077
X	" "	39	0.594	1.116	1.494	1.827	2.106	2.637	2.637	3.006	3.34	3.69	4.04	0.061

decoction medium was the lowest. If the growth rates at the end of 24 hours are computed from figures in tables 1 and 2, we have, as values for equal experimental periods, 0.225, 0.133, 0.147 for the cotton-, corn-, and potato-dextrose media, respectively. For this period growth on cotton-decoction medium is above that on potato-dextrose and corn-root decoction, while the difference between the growth on the latter 2 is considerable. Cotton-root-decoction medium is at least as favorable for the growth of strain 24 as potato dextrose and more favorable than corn-root-decoction medium. Computations of growth rates for strain 39 give 0.92, 0.061, and 0.146 for decoctions of cotton and corn roots and potato-dextrose medium. At the 24-hour period the values are 0.194, 0.076, and 0.137, respectively. For strain 39 the average growth rate during the 24-hour period and the longer experimental periods shows the same relationship; in decreasing order, cotton-root decoction, potato-dextrose, and corn-root decoction. The values show somewhat less variability than those obtained for strain 24. Corn-root-decoction medium was relatively less favorable than for the preceding strain, the growth rate on corn-root-decoction medium compared to that on potato dextrose being 89 per cent and 55 per cent for strains 24 and 39, respectively, at the 24-hour period.

From these experiments it is not evident whether the unfavorableness of the corn-root decoction is to be attributed to toxic substances or to a deficiency of nutrients available to the fungus. Succeeding experiments were carried out with freshly made decoctions of a concentration of 1:200. This liquid replaced the water in the usual potato-dextrose formula. Averages of results are given in table 3 and the curves of the values from experiment XIV are shown in figure 2.

It will be seen that corn-root decoction shows consistently the lowest growth rate, and cotton the highest. The average growth rates indicate the same relation, giving values for cotton, potato dextrose, and corn of 0.345, 0.102, and 0.015, respectively. Growth of hyphae on corn-root media developed a very distorted growth characterized by misshapen branches, or all growth ceased and the cells were plasmolyzed.

It is evident that even in the presence of an abundant supply of nutrients, corn-root decoction is an unfavorable medium for the growth of Phymatotrichum and that cotton-root decoction is highly favorable. The growth rate on potato-dextrose-corn-root-decoction medium is but 14 per cent of that on potato-dextrose medium, both media offering the identical nutrient conditions. The fresh corn-root decoction shows a much greater effect than the stored material used in experiment X.

Experiment XV was carried out with media that had been stored for 3 days under sterile conditions before beginning work. Both 24 and 39 were tested in these media and also in a fresh potato-dextrose-Turk's Cap-

TABLE 3.—*Growth of hyphal systems of Phymatotrichum omnivorum on potato-dextrose plus root decoctions*

Experiment	Media		Age of media	Strain	Average total growth in mm.				Average rate
					Hrs. 6	12	18	24	
XIV	Potato dextrose		fresh	39	0.918	1.638	2.043	2.448	0.102
	" "	cotton	"	39	2.790	4.590	6.480	8.280	0.345
	" "	corn	"	39	0.459	0.855	0.855	0.853	0.015
XV	Potato dextrose		3 days	24	0.405	1.485	2.475	3.402	0.141
	" "		3 "	39	0.945	1.395	1.575		0.087
	" "	cotton	3 "	24	0.720	0.945	1.080	1.530	0.063
	" "	"	3 "	39	0.450	0.990	1.395		0.077
	" "	corn	3 "	24	0.630	1.080	1.440	1.620	0.067
	" "	"	3 "	39	0.900	1.170	1.170		0.065
	" "	Turk's Cap	fresh	24	0.360	0.405	0.405		0.022
	" "	" "	"	39	1.440	1.170	1.710		0.095
XXII	Potato dextrose		fresh	24	0.765	1.350	2.250		0.125
	" "		"	39	0.720	0.990	2.925		0.162
	" "		"	Sc. cul.	2.745	3.905	8.10		0.450
	" "	wheat	"	24	0.540	0.540	0.540		0.033
	" "	"	"	39	0.450	0.945	2.205		0.122
	" "	"	"	Sc. cul	1.485	2.250	2.250		0.125
	" "	barley	"	24	0.675	0.990	1.710		0.090
	" "	"	"	39	0.620	3.060	4.680		0.260
	" "	"	"	Sc. cul.	0.720	0.720	0.720		0.036

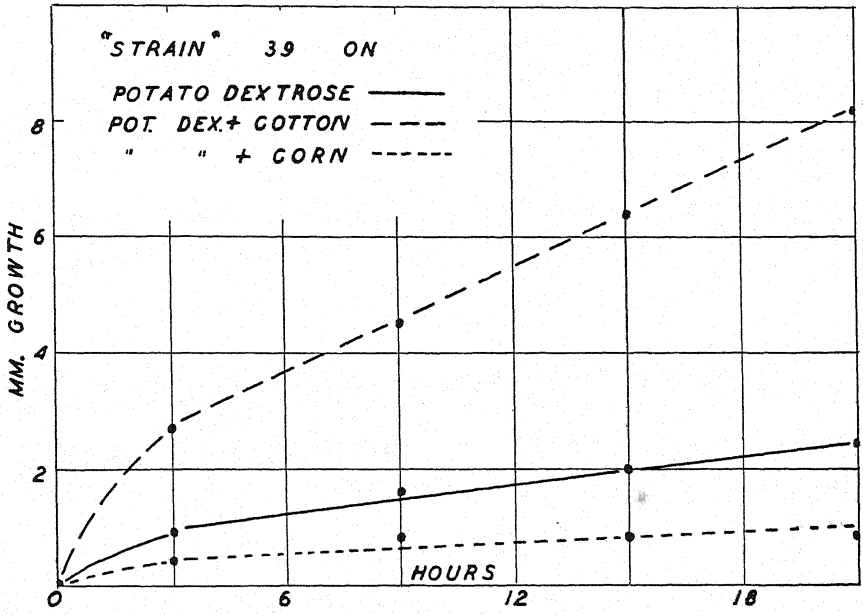


FIG. 2. Average rate of elongation of individual hyphae of "strain" 39 on various media.

root decoction, a plant unique among the Malvaceae in showing a high degree of immunity from root rot (1). In both strains, 24 and 39, the growth in cotton-decoction medium was less than that in potato-dextrose and very similar to that in corn-root decoction. Evidently a 3-day exposure under sterile conditions had largely eliminated the toxic effects of the corn-root decoction as well as the favorable effects of the cotton-root decoction. The 2 strains of Phymatotrichum reacted very differently to the fresh Turk's Cap-root decoction, 39 being unaffected and 24 markedly inhibited.

The same media were checked 3 days later in experiment XVI (not shown in table 3) when the decoctions of cotton and corn roots had been exposed for 6 days under sterile conditions and the decoction of Turk's Cap root 3 days. Strain 39 showed no significant difference in growth on the media, while strain 24 made poor growth on all the media.

The growth rates of strains 24 and 39 and a sclerotial culture were observed on freshly made potato-dextrose, potato-dextrose-barley-root decoction and wheat-root decoction. These monocots, like corn, are resistant to Phymatotrichum. The average values obtained are given in the final column, table 3. As in table 1, the sclerotial culture showed a much greater growth on potato dextrose than either 24 or 39, the average growth rates being 0.45, 0.125, 0.162 for the cultures in the order named above. Wheat-root decoction was markedly unfavorable, the average growth rates for

sclerotial culture and strains 24 and 39 being 0.125, 0.033, 0.122, respectively. The average growth rates in potato-dextrose-wheat-root decoction were 27, 26, and 75 per cent of those obtained in potato-dextrose for the sclerotial culture and strains 24 and 39, respectively. Barley-root decoction with potato dextrose showed a variable result, with the average rate of growth in the order named above being 0.036, 0.09, and 0.261. Sclerotial culture and strain 24 showed but 70 per cent of growth in this decoction compared to that observed on potato-dextrose medium; in contrast to this, strain 39 exhibited a growth on the barley-root decoction nearly twice that observed on potato-dextrose. The hyphae that grew on the decoctions of both wheat and barley roots exhibited the distorted hyphae previously described as those characteristically growing on the corn-root-decoction medium.

DISCUSSION

It will be seen in the final column of table 1 that, although there is considerable variation in the growth rates of a single strain, no trend toward lower values is evident. Thus, the pure cultures that furnished the inoculum for experiment I were maintained by repeated transfers for 6 months and used for experiment XXII. Although the growth rates for strain 24 fell from 0.22 to 0.125, the growth rate of strain 39 rose from 0.143 to 0.162; the lowest observed values for both strains occurring during the progress of this work were followed by high values. Similarly, the sclerotial culture showed no decrease in growth rate during the progress of these experiments. It would seem that during this period there was no decrease in vigor, as shown by the growth rate of any of the cultures.

As measured by growth rate there seems little difference between strains 24 and 39, the values for all experiments being 0.158 and 0.146, respectively. In contrast to this, the sclerotium culture showed a consistently higher rate of elongation. The difference in vigor of growth seems to be inherent in the different cultures, since it appears with different media and at different ages of the cultures. Although it is possibly modified by cultivation, the change is too slow to become evident in the present study. It is to be noted that the greater growth vigor shown by the culture from the sclerotium did not depend on the nutritional value of the sclerotium, since it was present in the culture only when the original isolation was made. Since the observed rates of growth for the sclerotial culture were 2 to 4 times those obtained for either strain, it seems doubtful whether this difference in the growth vigor is sufficient to indicate anything more than degree of activity that could hardly be assumed to have the permanence of a strain. It is evident that this sclerotium culture may be a third and conspicuously active strain, but this seems improbable. On the other hand, the reactions

of the cultures under other nutritional conditions seem to indicate that there is a real physiological difference.

All cultures show a decrease in growth vigor on potato dextrose 4 to 5 days after inoculation. Since, at this time, the fungus colony was not sufficiently large for food to be a limiting factor, it seems apparent that there are present in the medium certain staling products of metabolism produced by the fungus. These may be either carbon dioxide, ammonia, or both; these compounds have an inhibiting effect on the growth of the fungus (10).

In comparison with the growth rates obtained on potato dextrose, decoctions of cotton root proved a favorable medium. By the method of extraction with the dry ground root steeped in water at 100° C. and later autoclaved at 120° C., it does not seem probable that the medium contained an appreciable amount of protein. In the absence of a chemical analysis, it seems probable that the constituents must largely consist of soluble carbohydrates and mineral matter with a small amount of fatty acids and their derivatives and of amino acids. The acceleration of growth on the cotton-root-decoction medium must be due either to the presence of more readily available nutritive materials or to growth-promoting substances. This decoction medium is not favorable after aging, thus indicating that nutrient substances are not involved, since they would hardly disappear or oxidize to toxic substances during such a period.

This view is supported by the results obtained with a medium of potato-dextrose-cotton-root decoction. Growth on such a medium was far above that obtained on potato dextrose alone. This acceleration of growth lasts in such a medium until it has aged for several weeks.

A similar medium containing a decoction from corn roots was very toxic, causing a greatly distorted growth of the filaments and death. After this same medium had aged for several days, the growth rate on it equaled and in some cases excelled that on potato dextrose. This indicates the presence of certain labile water-soluble substances present in the roots of corn that are strongly toxic to the growth of the fungus. These substances in small quantities may act as a stimulant, as appeared in an experiment the results of which are shown in table 3. This is analogous to the stimulating effect of small doses of toxins on animals.

Certain water-soluble toxic materials appear to be present in the roots of Turk's Cap, and their action in causing the death of hyphae in contact with such a medium is similar to that observed in the corn-root-decoction medium. Whether these 2 roots contain similar substances that render them toxic to Phymatotrichum has not been determined, but it seems probable that this may prove to be the case. Decoctions of the same concentra-

tion of roots of barley and wheat had the same toxic effect, although it was not evident to the same degree in all cultures.

Susceptibility and resistance in species tried is paralleled by the growth-promoting and growth-retarding substances closely associated with nutritive values. These substances are more or less labile in solution. Though similar in physiological reaction, they are not identical, as may be inferred from the different degrees of toxicity and permanence in solution. In effective concentrations the mycelia stop growing or develop short, distorted branches and the protoplasm becomes vacuolate and finally becomes plasmolyzed and killed. The reaction of the fungus in the presence of these toxic substances is that of a parasite to unfavorable conditions. The vigor with which the fungus grows in the presence of these toxic substances gives a means of measurement of the effectiveness of the host's resistance to this parasite. The nature of these water-soluble substances present in the roots of resistant plants has not been determined, but it seems probable that they play an important rôle in establishing immunity. Whether the resistance of immune plants is solely due to the growth-inhibiting substances effective in decoctions cannot yet be definitely stated. They seem adequate, at least, to account for the resistant characteristics of monocotyledons.

Thus, it would seem probable that the rate of growth of hyphae of Phymatotrichum on decoctions of roots varies with the resistance of the plant to the parasite. Knowledge of the resistance of various species and varieties to the attack of this organism would be of great economic significance.

SUMMARY

A given culture of *Phymatotrichum omnivorum* grows at a fairly constant rate on potato-dextrose agar.

Different cultures vary in their vigor of growth on potato-dextrose agar.

A recently isolated culture averaged 2 to 4 times the rate maintained by cultures grown on artificial media for long periods.

Extracts from cotton roots furnish sufficient nutrient materials to sustain growth of the fungus. Cotton-root extracts added to potato-dextrose agar greatly accelerate the rate of elongation.

Extracts from corn roots when freshly prepared are strongly toxic to all strains of Phymatotrichum tested. Corn-root extracts in the presence of potato-dextrose agar have a similar effect on the fungus. Such water-soluble substances present in the roots of corn are labile but show a decreased toxic effect when aged.

Small doses of the toxins appear to be growth-stimulating.

Extracts from the roots of Turk's Cap, barley, and wheat all show a similar toxic effect but in varying degrees.

Evidently these water-soluble labile substances present in the roots of plants immune from the attack of Phymatotrichum have some part in establishing this immunity.

DEPARTMENT OF BOTANY AND BACTERIOLOGY,
UNIVERSITY OF TEXAS, AUSTIN, TEXAS.

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A SPHACELOMA ATTACKING NAVEL ORANGE FROM BRAZIL

ANNA E. JENKINS[1]

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INTRODUCTION

The Washington Navel orange (*Citrus sinensis* Osbeck), an introduction from Bahia, Brazil (3, 5, 10, 11, 12), is of great commercial importance in the United States (10, 11). In this country, together with other members of the *C. sinensis* group, it is highly resistant to or immune from attack by citrus scab (9, 14, 15), caused by *Sphaceloma fawcettii* Jenkins.

The present paper reports a disease caused by Sphaceloma, severely affecting the Bahia[2] Navel orange as grown in São Paulo, Brazil, together with studies leading to the identification of the pathogen in this genus.[3]

MATERIAL STUDIED

The specimens on which this study was based, contributed from São Paulo by E. E. Honey, consist of rind of ripe Bahia Navel orange fruits from Sorocaba (Fig. 1, A) collected on July 3, 1931, by F. S. José, and of rind of green fruits collected on Dec. 1, 1931, by C. B. P. Wright, at Limeira (Fig. 1, B), which is said (2) to be the most famous center of citrus production in the State.

NAMES AND IMPORTANCE OF THE DISEASE IN SÃO PAULO

According to E. E. Honey, the disease of the Bahia Navel orange discussed herein is referred to in São Paulo by the Portuguese names "*ver-*

[1] Thanks are due E. E. Honey, formerly of the Escola Superior de Agricultura "Luiz de Querioz," Piracicaba, Estado São Paulo, Brazil, for the specimens on which this study is based. For additional courtesies that have facilitated the work, acknowledgment also is made to others, among them P. H. Rolfs, Escola Superior de Agricultura e Veterinaria, Viçosa, Minas Geraes; H. S. Fawcett, of the Citrus Experiment Station, University of California; P. H. Dorsett, H. R. Fulton, and W. T. Swingle, of the Bureau of Plant Industry, United States Department of Agriculture.

[2] The varietal name "Bahia" as used in this paper or in any reference to the navel orange in Brazil relates to the variety of *Citrus sinensis* that is the original source of the Washington Navel orange of the United States. From plants of the Bahia variety that were introduced into the United States, the late William Saunders, Landscape Gardener and Superintendent of Gardens and Grounds of the United States Department of Agriculture, budded the 2 trees that were introduced into California in 1873 and from which developed (3) what may be termed the "Washington selection" of this variety, now known as the "Washington Navel."

[3] Reference to this paper is made in the U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. 16: 119. 1932 (Mimeographed).

rucose'' and *''sarna da laranga''* as well as by the English name ''citrus scab.'' In this paper it is referred to as ''navel-orange scab'' (p. 6).

E. E. Honey reports that in São Paulo navel-orange scab affects fruits of the Bahia Navel, often rendering them unmarketable, but that thus far the disease has not been observed on leaves and stems. For example, in an orchard entirely of this variety in which most of the fruit was unmarketable because of the severity of the disease, no trace of it could be found on leaves and stems. Again, as examined in a nursery the leaves of this orange were unaffected by this disease, while the leaves of sour orange, *Citrus aurantium* L., were severely affected by what is possibly the common citrus scab.

CULTURAL STUDIES

Tissue-culture isolations were made from the 2 specimens from Brazil. The Sphaceloma was slower in developing from the ripe fruits and few colonies were produced, but the cultures from the 2 lots of material were, otherwise, of essentially the same characteristics, as exemplified by figure 1, C and D. Compared in parallel cultures on test-tube slants of several different kinds of agar media, with representative cultures of Sphaceloma isolated from Citrus from other sources, the Brazilian fungus was clearly the most distinct of all. It showed a general resemblance, however, to the other cultures.

The 27-day-old cultures on wort (Fig. 1, C, D, L–P) and potato-dextrose (Fig. 1, E, Q–U) agar represent one of these comparisons. Of the wort-agar cultures, C and D are isolations from the Limeira and the Sorocaba specimens, respectively; L and M are from citrus grown in Florida. L (culture 8 A. E. Jenkins) was isolated from rough lemon, *Citrus limonia* Osbeck, in 1916 by H. E. Stevens, and M from Tahiti lime, *C. aurantifolia* Sw., in 1925 by the writer. The Stevens culture was previously employed to illustrate the wrinkled or convolute type of growth in *Sphaceloma fawcettii,* and the culture from Tahiti lime, the smooth or pulvinate type of growth in this fungus (7). In the order named, the isolations shown in N–P are from the following sources: N, from rough lemon grown in New South Wales;[4] O, from calomondin, *C. citis* Blanco, in Formosa,[5] and P, from sour orange, in the Union of South Africa.[6] In the comparison are also included 2 isolations of the Sphaceloma causing scab of avocado, *Persea americana* Mill., formerly confused (6) with *S. fawcettii.* One of these, shown in F on potato-dextrose agar and in H on wort agar, is from avocado grown in Florida and the other, shown in G on potato-dextrose agar and in I on wort agar, from avocado grown in the Union of South Africa.[6]

[4] Culture contributed by F. C. McCleery.

[5] Culture isolated by the writer from a specimen sent by G. Goto.

[6] Culture contributed by E. M. Doidge.

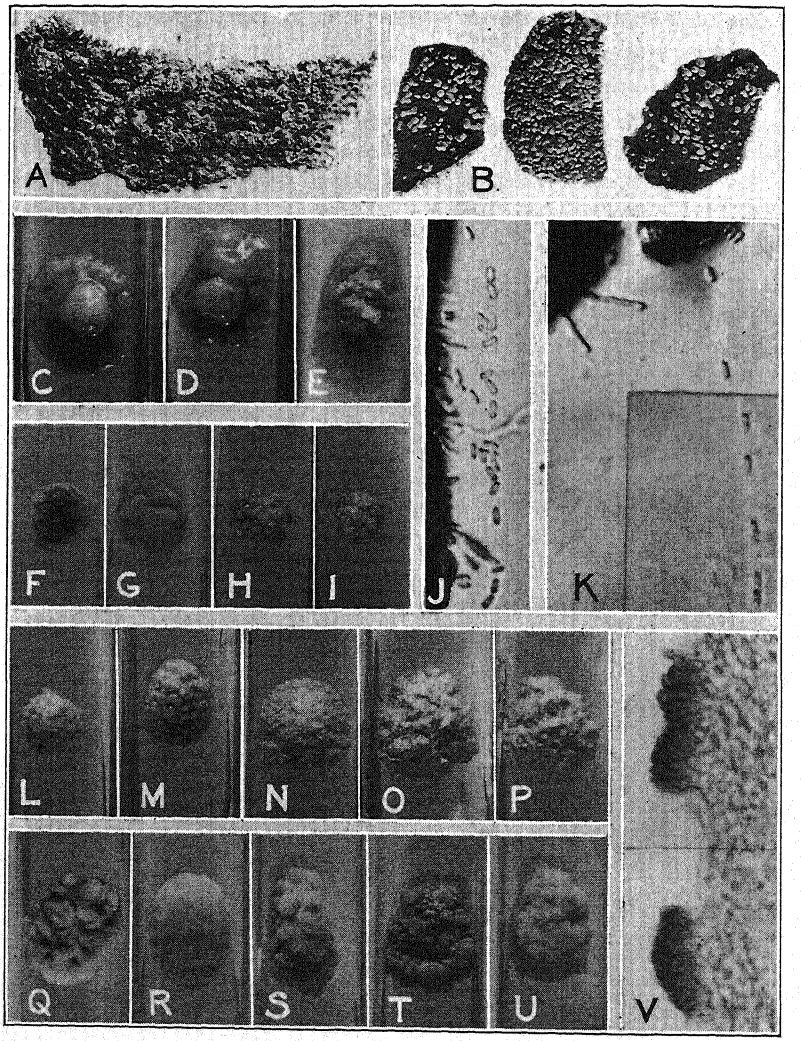


FIG. 1. A and B. Scab on rind of Bahia Navel orange from Sorocaba (A) and from Limeira (B). ×1. C–I, L–U. Comparison of navel-orange scab organism (C–D, on wort agar, and E, on potato-dextrose agar) with 7 other isolations of Sphaceloma, 2 from avocado (F and G, on potato-dextrose agar, and H and I, on wort agar), and 5 from citrus not grown in South America and not including *Citrus sinensis* (L–P, on wort agar, and Q–U, on potato-dextrose agar). All ×1. For further explanation, see text. J–K. Comparison of conidia of *Sphaceloma fawcettii* (J) and of the Sphaceloma causing navel-orange scab (K). Both ×300. V. Acervuli on lesion from specimen shown in B. ×380. V. Photograph by J. F. Brewer; the other photographs by M. L. F. Foubert.

Figure 1, C and D, shows the abundant viscid covering that has thus far been present on wort-agar cultures of the Brazilian Sphaceloma, even when several weeks old. In this same set of wort-agar cultures, observed at the time the photograph was made and a few days later, the 2 cultures from citrus grown in Florida, particularly the one from Tahiti lime (Fig. 1, M), as well as the Australian culture (Fig. 1, N), and one of the avocado cultures (Fig. 1, H), also were covered with a viscid substance. In all cases, however, this was present in a much smaller amount than in the Brazilian cultures. As these cultures aged, those of the Brazilian Sphaceloma became much darker than here represented and were free from the viscid covering. When 7 weeks old they ranged from Buffy Citrine[7] through Buffy Olive to Medal Bronze. The potato-dextrose-agar cultures shown in E and Q–U correspond to those on wort agar shown in D and L–P. On this substrate the Brazilian fungus was distinguished from the other cultures by the Light Orange-Yellow of the outer part of the culture. Later, the Light Grayish Vinaceous central part of this culture became black, while the other isolations remained light colored.

The cultures just described were grown at room temperature. The same isolations were compared in parallel cultures on potato-dextrose-agar test-tube slants at constant temperatures of 0°, 5°, 10°, 20°, and 25° C. For these comparisons, as for those shown in figure 1, the transplantings, from stock cultures, were about 0.5 mm. in diameter. At these several temperatures the Brazilian culture was again separable from all of the other cultures. In all cases essentially the same amount of growth was produced at a given temperature. In month-old cultures there was no growth at 0° C., slight growth at 5° C., and maximum growth at 25° C. During this period none of the viscid substance was observed on any of the cultures, although later a small amount developed about the margins of the cultures from Brazil and Japan.

Figure 1, J, illustrates conidial formation from small mycelial masses of the Stevens culture from Florida, approximately 22 hours after transplanted to corn-meal agar on glass slides. K represents a corresponding culture from the Sorocaba culture. As soon as made, the cultures were placed in Petri dishes lined with moist filter paper. It will be noted that the conidia in the Brazilian culture are of the same size or only slightly smaller than those of the Floridian, but that in the latter culture they are more abundant. In a similar set of equally old cultures, conidia were present in large numbers in both sets of cultures when first examined, but many of those of the Stevens culture had already become swollen or had germinated, while those of the Brazilian culture (Limeira isolation) were

[7] Color readings by J. Marion Shull, based on Ridgway, R., Color Standards and Color Nomenclature, 43 pp., 53 col. pls. Washington, D. C. 1912.

not noticeably so differentiated. Essentially the same conditions obtained when water was substituted for the agar medium.

DISTRIBUTION AND CLASSIFICATION

No definite record of the distribution of the Sphaceloma of the Bahia Navel orange outside of São Paulo is available. At Viçosa, Minas Geraes, as thoroughly examined in May, 1932, by P. H. Rolfs, who sent representative specimens to the writer, this variety was scab-free, while lemon, Rangpur lime, *Citrus aurantifolia,* bittersweet orange, *C. aurantium,* and Satsuma, *C. nobilis unshiu* Sw., were severely diseased.

In the State of Bahia, where it is believed (3) to have "originated as a sport, or mutation, upon a Selecta orange tree," *Citrus sinensis,* the Bahia Navel is apparently free from scab. In the paper just cited, Dorsett, Shamel and Popenoe discuss orange-tree diseases as observed by them while making a study of the Bahia Navel orange in Bahia and elsewhere in southern Brazil in 1913–14. No scab is mentioned, and numerous photographs of navel-orange fruits, including those published in the treatise just cited, are remarkably free from blemishes of any kind. In showing these photographs to the writer recently, Dorsett stated that he does not recall having seen scab lesions on Citrus while he was in Brazil during the period mentioned. Bondar (1, p. 70) has since reported scab in the State of Bahia on seeded oranges but not on the Navel. As of particular interest here, his discussion of the disease in this State, as well as those diseases in the South, is translated from the original as follows:

The disease may be considered as one of the most virulent citrus diseases. In Brazil it is very common in the States of Bahia, Rio de Janeiro, and São Paulo. The species most attacked are those with seeds, and in the first place, sour orange, Selecta, laranja de China (*Citrus sinensis*), lemon, pomelo, etc. The navel is not affected. It is not affected even when grafted on severely diseased sour-orange stocks. For this reason the disease is only of relative importance, since it affects only nurseries. Even so, it should be controlled. In the culture of seeded oranges, such as the Selecta and others, in the States to the South, it is necessary to avoid the propagation of the disease and to eradicate it wherever it appears. On the orange tree, as on the sour orange, it affects leaves, stems and fruits. The fruits lose all commercial value. In the State of Rio de Janeiro, the Minister of Agriculture has prohibited the entrance of any trees of the genus Citrus affected by scab.[8]

[8] The original text from which the translation is made is quoted below:

"*A molestia pode considerada como uma das mais virulentas no genero Citrus. No Brasil ella é muito commum nos Estados da Bahia, Rio de Janeiro e S. Paulo. As especies mais atacadas são as de carço, e em primeiro logar a laranja da terra, selecta e*

A quarantine measure,[9] dated as early as 1923, recognized the occurrence of scab in the Federal District and the municipality of Nictheroy, in the State of Rio de Janeiro (13).

It would hardly seem that the Sphaceloma of the Bahia Navel orange is identical with that affecting the common sweet orange in Bahia, for we have Bondar's (1) explicit, twice-repeated statement that at Bahia where the common sweet orange is affected by scab the navel is immune. It is possible that the scab of the sweet orange at Bahia is a form of the common citrus scab that has not yet attacked the navel. On the other hand, it is possible that scab of the sweet orange in Argentina, which G. L. Fawcett (4) states is distinct from citrus scab and is practically limited to fruits, may be the same disease as that of the Bahia Navel here reported. This disease has been referred to recently as sweet-orange scab (8).

Because it is highly pathogenic on the Bahia Navel and is culturally distinct from *Sphaceloma fawcettii*, which it resembles, the Brazilian Sphaceloma is classified as a variety of that species, *i.e.*, var. *viscosa*.[10] Additional studies[11] of Sphaceloma pathogenic on sweet orange other than

da China, limoeiro, pomelo, etc. A laranja de umbigo é indemne á esta molestia.'' [p. 70].

''A laranja de umbigo é imune á molestia. Mesmo sendo enxertada nos cavallos da laranja da terra muito atacados, ella não apresenta manifestação alguma deste mal. Devido a este facto a verruga, para a citricultura bahiana, tem uma importancia relativa, prejudicando sómente os viveiros. Nem por isso devemos descuidar de combater o mal.

''Para a cultura de laranja de caroço como selecta e outras, cultivadas nos Estados do Sul é de toda a necessidade evitar-se a propagação do mal e emprehender a extincção onde elle se manifestar. Nas laranjeiras, como na da terra o cogumelo ataca as folhas, os ramos e os fructos. As fructas doentes perdem todo valor commercial. Para o Estado do Rio de Janeiro o Ministerio da Agricultura prohibiu a entrada e a sahida de qualquer arvore do genero Citrus, atacada *por esta molestia.''* [p. 72–73.]

[9] This quarantine measure is quoted below:

RESOLVE, considerar zonos enfestadas pelo fungo Cladosporium citri Mass., doença das laranjeiras, cidreiras, etc., a Districto Federal e o Municipio de Nictheroy, no Estado do Rio de Janeiro, bem como que fiquem nas alludidas zonas, prohibido o transito e a exportação de plantas acima mencionadas, sem o certificado de sanidade, passado pelo encarregado official do Serviço de Vigilancia Sanitaria Vegetal.

Rio de Janeiro, 11 de Abril de 1923—Simões Lopes.

[10] A technical description of this new variety is given as follows:

Sphaceloma fawcettii var. *viscosa* n. var.

Acervuli (Fig. 1, V) on orange rind, dark colored, in section nearly Old Gold, 40 ± μ across, conidiophores 1–4 cells, 6–18 × 4–5 μ, and conidia (only a few seen) 6 × 4 μ. Hyaline conidia produced in culture of same measurements or somewhat larger or smaller. In culture distinguished from the species, particularly by its predominantly Light Orange-Yellow coloration as grown on test-tube slants of potato-dextrose agar; by the persistent viscid substance it produces on this substrate and especially on wort-agar test-tube slants and also by its coloration in old wort-agar test-tube cultures, in 7-week-old cultures, ranging from Buffy Citrine through Buffy Olive to Medal Bronze. In com-

the Bahia Navel in South America will doubtless assist in determining further the relationships of the organism. The term navel-orange scab is proposed to designate the disease caused by *S. fawcettii* var. *viscosa*.

In the United States, Peltier and Frederich (9), discussing the relative susceptibility of Rutaceae to scab in 1923, said that "on the whole . . . the sweet-orange group with an occasional exception, is nonsusceptible to scab." No scab was observed on the Washington Navel orange in Alabama where Peltier and Frederich's (9) studies extending over a considerable period were made. As a result of similar studies in Florida, Winston (14) and Winston, Bowman, and Bach (15) have reported essentially the same conclusions. Winston, Bowman, and Bach (15) say that "the common or sweet orange of commerce . . . is affected so rarely that for all practical purposes it may be considered immune. A trace of scab has been observed a few times on each . . . Florida Seedling, Lue, Maltese, Mediterranean Sweet, Pineapple, and Washington Navel." The fact that the sweet-orange group is essentially immune from scab in southeastern United States is an indication that the sweet-orange disease or diseases just discussed are not distributed there.

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parison with those of the variety, potato-dextrose-agar cultures of the species (culture 8 A. E. Jenkins) were Pale Grayish Olivaceous, and wort-agar cultures, more nearly Buff Pink.

Distribution: On fruits of Bahia Navel orange (*Citrus sinensis* Osbeck), Sorocaba and Limeira, São Paulo, Brazil.

Specimens and cultures examined: On Bahia Navel orange, São Paulo, Brazil, Sorocaba, July 3, 1931, E. S. José, and Limeira, Dec., 1931, Carlos Wright; cultures 345 and 356 A. E. Jenkins, isolated from the Sorocaba and Limeira specimens, respectively. Specimens and representative cultures, the specimen from Limeira designated as the type specimen, have been deposited in the Mycological Collections of the Bureau of Plant Industry, Washington, D. C. A living culture from the Sorocaba specimen (culture 345 A. E. Jenkins) has also been deposited in the Centraalbureau voor Schimmelcultures, Baarn, Holland, where a living culture of culture 8, referred to above, has already been deposited.

[11] Several months after this paper was written, specimens of Sphaceloma on sweet-orange fruits from Argentina were sent by G. L. Fawcett and also on sweet-orange fruits and sour-orange leaves, from São Paulo, by C. P. B. Wright, Director, Serviço de Citricultura, São Paulo. Judging from a preliminary comparison the sour-orange fungus is the same species as *S. fawcettii*. It produces the Cladosporium-like growth characteristic of this species (7). The Sphaceloma on sweet orange from Argentina and São Paulo, as well as that from Paraguay collected by Balansa in 1882 (7, 8), resembles *S. fawcettii* var. *viscosa*. Isolations from the recent specimens of sweet orange resemble cultures of *S. fawcettii* var. *viscosa* more closely than standard culture 8 of *S. fawcettii*, while those of the sour-orange fungus from São Paulo are more nearly like culture 8. Although they resemble each other as just described, all of these cultures are distinguishable from each other. On potato-dextrose agar the Argentine fungus produces Phoma-like structures containing micro-conidia of the same appearance as the conidia shown in figure 1, J and K, except that they are much smaller. A more or less similar stage has been observed for *S. fawcettii* var. *viscosa*, grown on the same substrate.

SUMMARY

On the basis of specimens received from São Paulo in 1931, the existence of a Sphaceloma highly pathogenic on the Bahia Navel orange in São Paulo, Brazil, is reported. The organism is culturally distinct from isolations of Sphaceloma from several kinds of Citrus (not including the *Citrus sinensis* group) grown in other countries, the United States, Japan, Australia, and South Africa, although in its temperature relations it appears to agree with them closely. It is classified as *S. fawcettii* var. *viscosa*. The disease it produces, termed navel-orange scab, appears to be limited to fruits, which it often renders unmarketable.

DIVISION OF MYCOLOGY AND DISEASE SURVEY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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THE QUINCE-RUST DISEASE CAUSED BY GYMNOSPORANGIUM GERMINALE

H. E. THOMAS

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INTRODUCTION

The morphological aspects and life cycle of the fungus *Gymnosporangium germinale* (Schw.) Kern have been ably treated particularly by Thaxter (17), who demonstrated the connection between the telial and aecial stages, and by Kern (12) in a monograph of the genus and elsewhere. The discovery of the wide prevalence of quince rust on the cultivated apple (9, 11, 14, 18) has pointed to the need for more exact knowledge of many other phases of the disease.

PLANTS AFFECTED

Kern (12) in 1911 recorded the telial stage on *Juniperus communis* L., *J. sibirica* Burgsd., and *J. virginiana,* L., and no additions to the suscepts for this stage seem to have been made.

In addition to the species of Amelanchier, Aronia, Crataegus, Cydonia, and Malus listed by Kern (12) as susceptible, a number of species of Crataegus have been added more recently (1, 13). The writer has collected the fungus on 14 species of Crataegus[1] in central New York, the following 7 of which have not been found in earlier lists: *C. Arnoldiana* Sarg., *C. beata* Sarg., *C. calpodendron* (Ehrh.) Medic., *C. brainerdi* Sarg., *C. filipes* Ashe, *C. holmesiana* Ashe, *C. monogyna* Jacq. The fruits of *C. oxyacantha* L. were rather severely affected by the disease at 2 stations in the outskirts of Ithaca in 1931. Farlow in 1880 (6) recorded *Roestelia aurantiaca* on *C. oxyacantha.* A collection of this fungus on *C. oxyacantha* in the Herbarium of the Department of Plant Pathology of Cornell University was made by H. H. Whetzel, on the University Campus, in 1902.

VARIETAL SUSCEPTIBILITY

While varieties have not been generally recognized in the red cedar, it is a matter of common observation that individual trees may vary rather widely in appearance when grown side by side. It has been observed, furthermore, that different cedar trees equally exposed to inoculation may be quite unequally affected by this disease and, likewise, by the apple and hawthorn rusts caused by *Gymnosporangium juniperi-virginianae* Schw. and *G. globosum* Farlow, respectively (10, 16, 19). This variation in susceptibility is concretely illustrated by the figures in table 1. Two localities

[1] The writer is indebted to Dr. K. M. Wiegand for the identification of the species of Crataegus.

and groups of trees are represented in the table, one by the first 2 trees and the other by the last 3. In each group, the trees are about equally exposed to inoculation by the quince and hawthorn fungi. The nearest source of the apple-rust fungus is somewhat more distant (30 to 40 ft.) for the first group, and no near-by source of this fungus is known for the second group.

TABLE 1.—*Variation in susceptibility of cedar trees to the rusts of apple, hawthorn, and quince*

Tree	Percentage of total infections			Number of infections
	Apple rust	Hawthorn rust	Quince rust	
1	68	32	0	50
2	12	2	86	50
3	0	67	33	200
4	0	7	93	200
5	0	71	29	200

Temporary variations in the susceptibility of trees cannot be invoked as a plausible explanation of the observed facts, since the quince-rust fungus is perennial on the cedar over a long period of years.

Sufficient information is not yet available to permit of a detailed classification of apple varieties as to susceptibility even for a single locality. That a wide variation among varieties does exist, however, is readily apparent. The following figures represent the maximum percentages of fruit infection that have been recorded for several of the more common varieties in Hudson Valley orchards: Delicious 65, Fameuse 21, Hubbardston 28, Jonathan 22, McIntosh 59, Northwestern Greening 24, Winesap 76. Rome Beauty was affected in 41 per cent of the fruits in 1 orchard near Geneva in 1930. In contrast, none of the following varieties has yet been found in the State with as much as 5 per cent of quince-rust infection on the fruit: Baldwin, Ben Davis, Northern Spy, Rhode Island Greening, Twenty Ounce, and Wealthy.

SYMPTOMS

The principal symptoms of the disease on the various affected plants have been described by Weimer (19), Dodge (4), Thomas and Mills (18), and others. A few additional features may be worthy of mention.

On the cedar there is at times a tendency toward the production of small but definite witches' brooms, a characteristic conducive to confusion with the related disease caused by *Gymnosporangium Nidus-avis* Thaxt. Also, on the cedar are found occasionally the galls produced by *G. globosum* partially or largely preempted by *G. germinale* and continuing to produce telia of both species. In such cases the telia of *G. germinale* are usually evident also on the adjacent stems.

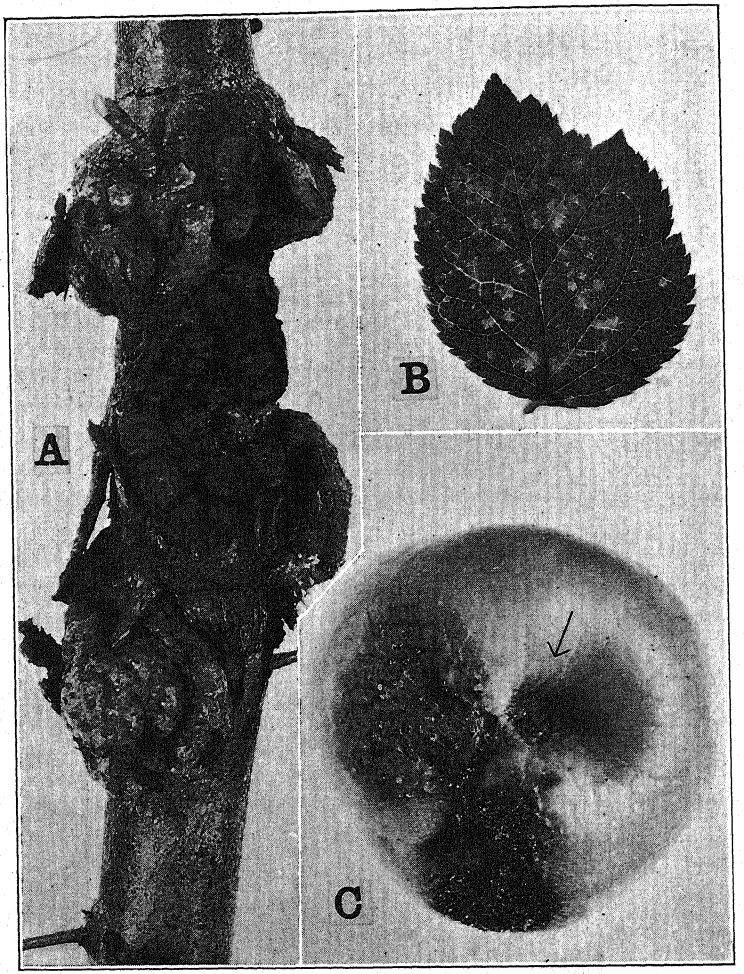


FIG. 1. Symptoms of quince rust. A. Perennial infection on stem of *Crataegus holmesiana*. B. Infection on seedling apple leaves inoculated artifically. C. Necrotic quince-rust lesion (indicated by arrow) and 2 apple-rust lesions on Rome apple fruit.

The disease produced by the aecial stage of the fungus is primarily one of fruits and twigs. On certain susceptible species of Crataegus, the larger stems may be invaded. (Fig. 1, A.). By artificial inoculation, Farlow (7) obtained infection and pycnia on seedling apple leaves (presumably with *Gymnosporangium germinale*) and Miller (15) has had similar results with quince foliage. The writer has obtained heavy infection on leaves of potted plants of *Crataegus* spp. with numerous pycnia, but aecia were produced only sparingly along the larger veins. Chlorotic spots were also produced on seedling apple leaves (Fig. 1, B), but these never developed pycnia and

gradually became less distinct. Similar but still less marked symptoms have been produced on a few inoculated leaves of McIntosh apple.

The symptoms on 24 varieties of apple fruits that have been examined are predominantly necrotic or hypoplastic and rarely hyperplastic. Pycnia when present are individually more prominent than those of the apple-rust fungus but are more widely scattered, rarely, if ever, resulting in the bright orange color that is characteristic of the apple rust. The absence of the orange color, however, is not a reliable diagnostic symptom of quince rust, for apple-rust lesions are sometimes seen even with mature aecia and yet with no visible orange color. In situations favorable for both of these diseases, it is not uncommon to find infections of each on the same fruit, (Fig. 1, C).

ETIOLOGY

The reported differences in susceptibility to apple rust of the same varieties grown in different localities are shown by the work of Bliss (2) to be due in part to distinctly different strains of the fungus. A few infection experiments bearing on the question of pathogenic strains have been made with the quince-rust fungus (all collections from central New York). On 4 occasions, single Crataegus plants were inoculated on opposite halves with telial material from different sources. Abundant infection was obtained in all cases but on 2 plants the later development of the infection seemed more vigorous on one half of the plant than on the other. Again, on 2 different dates, single seedling apple trees were inoculated with spores from one source and later when new leaves had developed the same trees were inoculated with spores from another source. In each instance one of the contrasted inoculations resulted in numerous infections (a maximum of 50 to 90 spots per leaf), while the other was followed by few or none. While these results are not regarded as conclusive evidence of pathogenic strains, it seems probable that more striking differences will be found when collections from more widely separated localities are studied.

An interesting feature in the life history of the fungus has been noted during the past year. In the autumn of 1930, a group of several plants of *Crataegus holmesiana* were found to be rather severely affected by quince rust in the twigs and stems. Several of these were marked and watched during the spring and summer of 1931. Several of the infections persisted through the winter and, judging from appearance and rate of development, some of them probably have survived 2 or more winters. (Fig. 1, A). Dodge (3) has mentioned similar cases of perennation in the aecial stage for *Gymnosporangium Botryapites* (Schw.) Kern and *G. transformans* Ellis.

Germination of Teliospores and Basidiospores.—Weimer (19) states that the germination of teliospores and basidiospores of *Gymnosporangium germinale* is in all respects similar to that described for *G. juniperi-vir-*

ginianae with an optimum temperature (for the teliospores) at 22 to 24° C. In the course of this work several tests have been made mostly at room temperature (about 25° C.) in an effort to learn something of the maximum rate of germination. Under the most favorable conditions that were provided, telia that were immersed in water for 25 minutes and removed to a relatively moist atmosphere, discharged basidiospores in considerable numbers within 2 hours after removal from the water. When dry telia were removed from the substrate, mounted on moist cotton and held at 25° C. there was found after 8 hours an abundant germination of teliospores and the beginning of basidiospore germination. Basidiospores submerged in water at room temperature were observed to develop germ tubes equal in length to the diameter of the spore within 2 hours and 4 to 6 times that length in 10 hours. A similar rate of germination was noted when basidiospores were mounted on a moistened Crataegus leaf and held at 25° C., the germ tubes reaching 5 to 7 diameters in length in 8 hours. It was not determined with certainty whether or not penetration of the leaf had taken place.

Germination of Aeciospores.—Earlier workers experienced difficulty in germinating the aeciospores of *Gymnosporangium germinale* and many other species of the genus. Doran (5) records minimum, optimum, and maximum temperatures for the germination of aeciospores of *G. germinale* at 9°, 16°, and 29° C. The actual percentages of germination are not shown. Thomas and Mills (18) report moderate germination at temperatures of 6° to 21° in spores from fruits previously held at 3°. More recently the writer has obtained up to 49 per cent germination in spores collected November 26, 1930, and kept over night at 3° and a maximum germination of 60 per cent in spores from fallen Crataegus fruits collected on January 22, 1929. However, on March 13, 1929, no viable spores were found in fruits from the same source. On April 4, 1931, a trace to 10 per cent germination was obtained in spores from mummied Crataegus fruits that had remained attached to the tree. These spores, however, were found only in deep-seated aecia with little or no opening to the outside.

In order to test further the influence of previous chilling on the germination of aeciospores, affected quince fruits were placed in open paper bags at 3° and 6° C. on October 1, 1930. Spores from these were set to germinate at 15° on 4 different dates from October 23 to November 13. The averages (each representing 4 counts of 100 spores each) for the spores from the sample held at 3° were 38.7, 28.5, 34.0, and 18.2 per cent, while those for the spores kept at 6° were 22.7, 19.2, 21.0, and 19.5. The relatively small difference between the results from the 2 samples may be due in part to the more rapid drying at the higher temperature.

It was assumed in the earlier tests that precooling was important in inducing the comparatively good germination that was reported. This

appears to have a foundation in the results of a test in which spores were mounted in water and after 25 hours at 6° to 8° C. were moved to 26°. Here a germination of 39 per cent was obtained (200 spores) as compared with 9.5 per cent (800 spores) for spores from the same source placed immediately at 26° without precooling.

However, the best germination in any of the writer's tests was that in spores mounted at 18° C. without precooling. The spores were taken on May 27 and June 20 from a Crataegus plant inoculated in the greenhouse on March 24. Aecia were first seen on this plant on May 23. In these 2 tests a total of 800 spores averaged 68.8 per cent germination with several counts of above 90 per cent. The same number of spores in parallel tests at 26° C. averaged 9.5 per cent germination.

It has been pointed out that the only viable aeciospores found by the writer under natural conditions in late winter were in deep-seated aecia with little or no opening to the outside. It is of interest then to know whether spores that are more exposed to atmospheric influences will survive for any considerable period. With this in mind, aeciospores were mounted on dry glass slides and placed in dry Petri dishes at 3° C. These were removed at intervals and tested for germination at 15° C. The average percentage germination of the spores was 34 at the beginning of the experiment, 18.7 after 2 days, 16.2 after 6 days, and 4.0 after 16 days.

In 2 tests, an attempt was made to detect any influence upon germination due to the presence of cedar leaves, either whole or macerated, in the drops of water. None was found in the 3,600 spores counted. On 2 occasions, May 28, and August 9, 1931, spores were seen (in free-hand sections) to have germinated in droplets of water in the axils of young leaves of potted cedar trees. In the first instance germination was abundant at the end of 25 hours and some evidence was seen of penetration by the germ tubes into the epidermis of the plant. However, this critical point must remain unsettled until a more detailed study is made.

Incubation.—Farlow (7) records the appearance of pycnia on apple-seedling leaves 10 days after inoculation with spores of *Gymnosporangium germinale.* In 4 infection experiments with relatively resistant foliage of apple seedling trees the writer was unable to detect any symptoms until 10 to 18 days after inoculation, and pycnia were never found on these. In contrast in 5 similar tests with susceptible foliage of Crataegus plants, symptoms were seen in 4 to 6 days, and pycnia appeared in 13 to 15 days after inoculation. These plants were grown in a greenhouse in which the temperature is partially controlled at 65 to 70° F.

Relation of Age of Tissues to Infection.—In the spring of 1931, inoculations were made at Ithaca on a few McIntosh fruits on each of 7 dates from May 7 to June 21, beginning when the buds were in the closed-cluster stage. In some cases the moistened telia were suspended over the parts to be inocu-

lated and in others moist cotton was brushed over the germinating teliospores and placed directly on the receptacle of the flowers or the young fruits. Some were covered with celophane bags and others were not. Rain and atmospheric moisture were depended upon for the maintenance of humidity, and these apparently were not adequate, for no infection resulted in any case on the fruit and nothing conclusive was seen on the foliage.

A more successful outcome followed the inoculation of Crataegus leaves in the greenhouse (Table 2). The inoculum was applied copiously to each leaf with a camel's-hair brush and the plants were maintained in a moist atmosphere for 3 days. Infection was so heavy as to preclude the counting of spots. It will be seen, however, that here, as in the case of apple rust on apple foliage (8), the leaf is susceptible in the earlier stages and gradually passes to a condition of complete immunity.

TABLE 2.—*Relation of age of leaf to infection of Crataegus by Gymnosporangium germinale. Records on potted plants 16 days after inoculation*

Branch number	Leaf number (oldest at left)																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Plant 1																									
1	0[a]	0	0	0	0	0	0	0	+[a]	+	+	+	+	+	+	+	+	+	+	+	+	0[b]	0	0	0
2	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0[b]	0	0	0					
3	0	0	0	0	+	+	+	+	+	+	+[b]														
4	0	0	0	0	0	+	+	+	+[b]																
Plant 2																									
1	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+[b]	0	0	0				
2	0	0	0	0	0	+	+	+	+	+	+	+	0[b]												
3	0	0	0	0	0	+	+	+[b]																	

[a] 0 = no infection; + = infection.

[b] Youngest leaf at time of inoculation.

SUMMARY

Plants of the following species of Crataegus not found in earlier lists are reported as affected by quince-rust: *Crataegus arnoldiana, C. beata, C. brainerdi, C. calpodendron, C. filipes, C. holmesiana, C. monogyna.*

Marked variation in the susceptibility of individual cedar trees is shown. Trees susceptible to the quince-rust disease may be relatively resistant to the apple or hawthorn rust.

Basidiospores may be discharged within 2½ hours from the time the telia are moistened. Basidiospores may begin to germinate within 2 hours after they are immersed in water.

Viable aeciospores are found occasionally in nature as late as April (at Ithaca) but in such situations as to render dissemination unlikely.

Good germination of aeciospores is obtained at 15 to 18° without having been preceded by any prolonged rest period.

Symptoms are seen 5 to 8 days after inoculation on foliage of susceptible Crataegus plants. The incubation period in comparatively resistant apple leaves is considerably longer.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

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PHYTOPATHOLOGICAL NOTES

A Destructive Fusarium Wilt of Muskmelons.[1]—In June, 1932, the writer's attention was called to a 6-acre field of muskmelons in Hennepin County, Minnesota, in which more than half of the plants had died within 2 or 3 weeks after emerging from the soil. Many of the hills had been replanted, and at the time of examination the seedlings of the second planting were just emerging from the soil and many of these also were wilting. There were no obvious lesions on some of the wilted plants, although there were definite reddish brown necrotic lesions on the roots and hypocotyl of many of them. On some of the lesions small pinkish spore masses were evident. Microscopic examination revealed these to be spores of a species of Fusarium. Isolations from a considerable number of wilted plants yielded in all cases the same species of Fusarium, which subsequent inoculation experiments proved to be the cause of the disease. When this fungus was compared with a culture of *Fusarium niveum* E. F. S. isolated from wilted watermelons[2] they were found to be very similar in morphological and cultural characters. When grown on several different kinds of media the red to purple pigment produced by the culture from muskmelon was slightly less intense than that produced by the culture from watermelon. The former culture also tended to produce fewer macroconidia and more microconidia than the latter. One hundred tricellular macrospores of each culture produced on potato-dextrose agar were measured. The length of the spores of the muskmelon culture ranged from 24 to 45 μ, while those of the watermelon culture ranged from 27 to 60 μ. There was no appreciable difference in the width of spores of the 2 cultures.

In spite of the close similarity of the 2 fungi in cultural and morphological characters, they differ strikingly in pathogenicity. Watermelons are highly resistant to the culture isolated from wilted muskmelons, while muskmelons are highly resistant to the culture causing wilt of watermelons.

The wilt of muskmelons was found in several fields in which were planted 1 or more rows of watermelons. In these fields more than 95 per cent of the muskmelons were killed by wilt, but not a single watermelon plant was injured. One such field is shown in figure 1, C. These observations were substantiated by greenhouse tests in which Kleckly Sweet watermelons and Golden Osage muskmelons were planted in pots of soil infested with the respective organisms. In these experiments the muskmelons were highly resistant to *Fusarium niveum* and completely susceptible to the culture ob-

[1] Paper No. 1171 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] This culture was kindly furnished by Mr. J. J. Wilson and was isolated from wilted watermelons in 1932 in Conesville, Iowa.

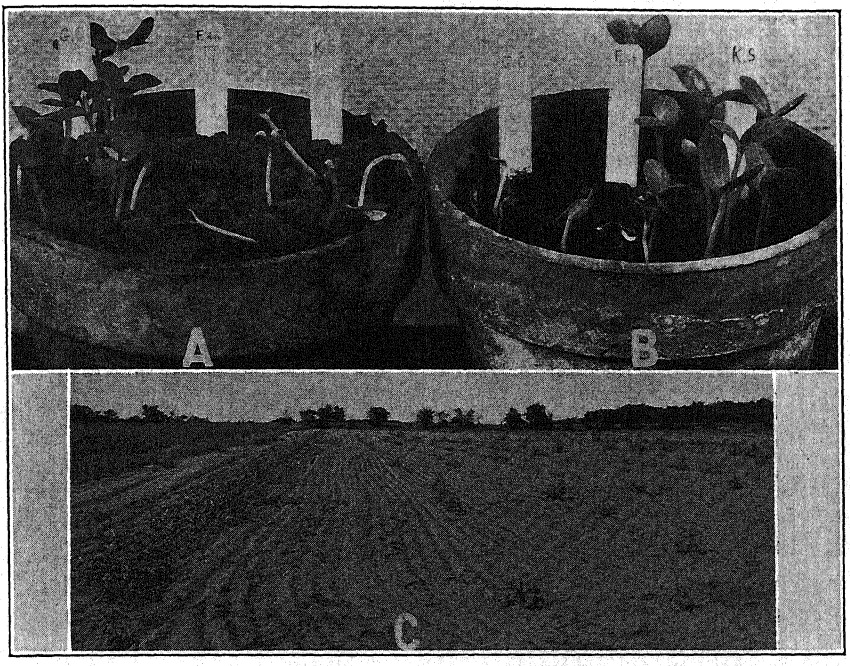


FIG. 1. A and B. Golden Osage muskmelons (G. O.) and Kleckly Sweet watermelons (K. S.) growing in soil infested with *Fusarium niveum* (A) and the Fusarium causing muskmelon wilt (B). C. Field infested with organism causing the wilt shown in B. Note that more than 90 per cent of the muskmelons have been killed by this wilt, whereas none of the watermelons in the single row at the left have yet been killed.

tained from muskmelons. On the other hand, watermelons were highly resistant to the culture from muskmelons and completely susceptible to *F. niveum* (Fig. 1, A and B). These results would indicate that the wilt of muskmelons may be caused by a physiologic form of *F. niveum* or by a closely related species.

A search of the literature has yielded very little definite information about Fusarium wilt of muskmelons. In 1898, Sturgis[3] observed a Fusarium fruiting on the stem of a wilted muskmelon in a field where bacterial wilt was prevalent, and, although no inoculation experiments were made, he suggested that "the wilt disease which has been so prevalent lately in our muskmelon fields may be due in part to the attacks of a species of Fusarium possibly the same as that associated with the wilt of watermelons."

The same year, Selby[4] reported a wilt of garden cucumbers, Japanese climbing cucumber, and muskmelon, and stated that it appeared "to be due

[3] Sturgis, William C. Some common diseases of melons. Conn. Agr. Expt. Sta. Ann. Rpt. 22 (1898): 225–235. 1899.

[4] Selby, A. D. Further studies of cucumber, melon and tomato disease, with experiments. Ohio Agr. Expt. Sta. Bul. 105. 1899.

to the same or to a similar fungus to that causing the southern watermelon wilt described by E. F. Smith." Here also no inoculations were made. Taubenhaus,[5] in 1920, published the results of his investigations of the wilts of watermelons and related crops. In numerous field trials on "watermelon-sick" soil he showed that cantaloupes remained 100 per cent healthy, while watermelons were 69 to 98 per cent killed. Taubenhaus made no mention of a wilt of cantaloupes in this study.

Stone,[6] in 1911, made brief mention of a Fusarium stem rot of cucumbers and muskmelons in the greenhouse but made no inoculations and did not identify the pathogen. It is not probable that he was dealing with the wilt in question, because the greenhouse tests and field observations show that cucumbers are not affected by the wilt of muskmelons occurring in Minnesota.

Cook[7] in 1923, reported a greenhouse disease of melons from which he isolated a Fusarium "very similar to *F. vasinfectum.*" No inoculations were made to determine the pathogenicity of the culture, and the disease is not described in sufficient detail to justify conclusions as to its identity.

Chupp[8] has reported a Fusarium wilt of muskmelon causing a trace to one per cent loss in several fields in New York. This may be the same as the wilt in Minnesota, but it was not described in sufficient detail to justify conclusions.

Although the wilt of muskmelons observed in Minnesota may kill a high percentage of the plants in the seedling stage, plants of any age may be affected. In 2 fields kept under observation additional plants died throughout the season until the occurrence of frost. Plants in later stages of maturity affected with wilt are characterized by brown necrotic streaks beginning at or below the ground line and extending from several inches to 2 ft. or more along 1 side of the stem. On such lesions masses of salmon pink spores may be produced in abundance.

The experience of several melon growers would indicate that the disease has been present in the State for at least 3 years. Records of the Department of Plant Pathology indicate also that it was observed in 1931.

The disease is being studied further and efforts will be made to select wilt-resistant varieties.—J. G. LEACH, University Farm, St. Paul, Minn.

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[5] Taubenhaus, J. J. Wilts of the watermelon and related crops. Texas Agr. Expt. Sta. Bul. 260. 1920.

[6] Stone, G. E. Fusarium disease of cucumbers and other plants. Mass. Agr. Expt. Sta. Ann. Rpt. 23 (Part II): 62–65. 1911.

[7] Cook, Mel. T. A greenhouse disease of melons. Phytopath. 13: 462–463. 1923.

[8] Chupp, Charles. Fusarium wilt of muskmelon. Plant Disease Reporter 14: 160. 1930.

Physiologic Forms of Colletotrichum falcatum Went.—The sudden failure of the sugar-cane variety P.O.J. 213 in Louisiana during the years 1930 and 1931 due to red-rot suggested the activity of physiologic forms of the causal organism (*Colletotrichum falcatum* Went) as a possible explanation. While thus far a detailed study has not completely explained that failure, definite evidence of the existence of physiologic forms has been obtained.

Many hundreds of isolations from rotting cane and leaf midrib lesions in Louisiana, Georgia, and Florida have been compared in culture and in pathogenicity on different varieties of cane. For the latter purpose stalk inoculations have been used. Briefly, the method followed consisted in making 20 inoculations per isolate in uniform material by placing the fungus in cork-borer holes in alternate internodes of the cuttings. These were incubated for 15 days in moist, steamed sawdust at 20–25° C., after which they were split and the spread of the fungus measured. In order to obtain reliable comparisons of large numbers of isolates, it was necessary to express the results mathematically. This was done by calculating the ratios of lateral and longitudinal spread of the lesion to the varying dimensions of the inoculated internodes. Thus, a numerical designation of 1.00 would indicate that the disease had spread the entire length (or width) of the internode, while values of less or more than 1.00, the proportionate parts of the same or adjoining internodes, respectively. By multiplying the ratio for length by that for width a figure is obtained which for convenience in comparing isolates has been termed the "index of virulence."

A representative series of inoculations is presented in table 1 comparing the indices of 4 isolates on three varieties of sugarcane. L–7 was obtained from rotted seed cane of P.O.J. 213 in Louisiana, F–1 from the same variety in Florida, and G–2 from rotted Cayana cane in Georgia. These cultures are representative of large populations isolated from these localities. L–31 was isolated once from severely rotted banked cane of Co. 281 in Louisiana.

The results in the table, which are similar to those from many other tests, show that L–7 and F–1 have approximately the same indices and should be considered as belonging to the same physiologic form. L–31 is more virulent on all three varieties than L–7, F–1 or G–2, its greater virulence on Co. 281 being particularly significant since this variety has been resistant to all other isolates obtained. Because of its greater virulence it has been considered a separate physiologic form, even in the absence of population studies. G–2 is weekly parasitic in comparison with the others and is classed as a third physiologic form.

It has been found that sugarcane varieties possess two kinds of resistance to *Colletotrichum falcatum*: (1) Functional, possessed by those which germinate quickly and are vigorous root formers, thus preventing

TABLE 1.—*Comparative virulence of four isolates of Colletotrichum falcatum on three varieties of sugarcane*

Culture No.	P.O.J. 213				C.P. 807				Co. 281			
	Length	Diameter	Index of virulence	Relative virulence L–7 = 100	Length	Diameter	Index of virulence	Relative virulence L–7 = 100	Length	Diameter	Index of virulence	Relative virulence L–7 = 100
					1st Series							
L–7	1.74	0.97	1.69	100	1.34	0.75	1.00	100	1.01	0.65	0.66	100
F–1	1.98	0.89	1.76	104	1.36	0.69	0.94	94	0.96	0.74	0.71	108
L–31	1.98	1.00	1.98	117	1.41	0.81	1.14	114	1.51	0.91	1.37	208
G–2	1.43	0.87	1.24	73	1.03	0.65	0.67	67	0.64	0.40	0.26	39
					2nd Series							
L–7	1.10	0.68	0.75	100	0.97	0.62	0.60	100	0.54	0.52	0.28	100
F–1	0.94	0.78	0.73	95	0.99	0.70	0.69	115	0.56	0.46	0.26	108
L–31	1.73	0.89	1.54	205	1.31	0.84	1.10	183	0.97	0.73	0.71	254
					3rd Series							
L–7	1.07	0.68	0.73	100	0.97	0.64	0.62	100	0.53	0.40	0.21	100
F–1	0.94	0.74	0.70	96	1.00	0.65	0.65	105	0.56	0.45	0.25	119
L–31	1.73	0.88	1.52	208	1.31	0.45	1.05	169	0.98	0.69	0.68	324

the entrance of the fungus through the root primordia; and (2) physiologic, in which the development of the fungus within the tissues of the stalk is retarded once infection has taken place.—E. V. ABBOTT, Division of Sugar Plant Investigations, Bureau of Plant Industry, Houma, La.

Authority for the Name Cronartium ribicola.—The name of the white-pine blister rust has generally been accredited to Fischer by American authors and to Dietrich by European authors. The writer is clearly responsible for introducing the usage prevalent in America, and possibly for the one exception to the usage in Europe, occuring in Grove's British Rust Fungi.[1]

In the North American Flora, vol. 7,[2] the name was given as Fischer von Waldheim. Not long after the publication of that part of the Flora in 1907 Perley Spaulding visited the herbarium at La Fayette and desired to know the grounds for this selection of authority. It was recognized at the time that Dietrich's name of 1856 (or possibly 1854) was a *nomen nudum,* while Fischer[3] in 1872 had published a description of *Cronartium ribicola* Fischer, nov. sp., and also issued a collection under this name in Rabenhorst's Fungi Europaei Exsiccati, No. 1595. This collection, as represented in the Farlow Herbarium, shows that Fischer had the right fungus, for it bears both urediospores and teliospores. This fact does not, however, affect the nomenclature. The full name of this "Fischer" was not stated at the time, and it remains unknown up to the present, even after much effort expended by the writer and others. The name of Fischer von Waldheim was familiar to American mycologists from the publication of a translation of his extensive paper on the "Biology and history of the development of the Ustilagineae," and it was easy but indefensible to assume the identity of the two Fischers. Although the error was at once recognized it was not convenient to make a correction until the 10th part of the Flora[4] was reached in 1925.

Perley Spaulding[5] has called attention to the unsatisfactory usage by American authors and indorses the conclusion reached by Klebahn[6] in his extended study of the matter in which all the data known up to the present time are carefully considered.

[1] Grove, W. B. The British fungi. 412 pp. University Press, Cambridge. 1913. (p. 316.)

[2] Arthur, J. C. North American Flora 7: 122. 1907.

[3] Fischer. *Cronartium ribicola* Fischer nov. sp. Hedwigia 11: 182. 1872.

[4] Arthur, J. C. *L. c.,* p. 692. 1925.

[5] Spaulding, Perley. Dietrich preferable authority for *Cronartium ribicola.* Phytopath. 23: 203–204. 1933.

[6] Klebahn, H. Der Autor des *Cronartium ribicola.* Ztschr. Pflanzenkr. 41: 209–213. 1931.

The question to be settled is seemingly very simple. Should the name be written *Cronartium ribicola* Dietrich or *Cronartium ribicola* Fischer, as these are the only authors who have ever referred the fungus to the genus Cronartium? The undisputed facts are these: Dietrich used the name in a local flora, published no description, claimed that it was a new species, and issued in a published exsiccati a specimen that bore teliospores. Fischer published a description, called it a new species, and also issued a specimen in a published exsiccati. In accordance with the International Rules of Nomenclature it must be admitted that Dietrich's name in these respects was by itself, and still is, a *nomen nudum,* while Fischer's name was duly established. One cannot, therefore, agree with the conclusion reached by Klebahn, following an extended and ingenious argument, that: "One thus leaves to Dietrich the honor of being the author of the fungus, since he discovered it, correctly named it and distinguished it, as well as is generally possible without knowledge of the aecidia, as a Cronartium which lived on Ribes." If this procedure were generally adopted, a *nomen nudum* would no longer be a barrier to the acceptance of a name as valid.

But this is not the whole story. Neither Dietrich nor Fischer originated the specific name *ribicola,* but a much earlier author, G. W. Lasch, who published the name in 1842 under the genus Uredo, with a description and specimen in Klotzsch's Herb. Myc. 490. This name, *Uredo ribicola* Lasch, according to Klebahn, cannot be considered in priority, because "teleutospores were not observed." The description was reprinted in Rabenhorst's Deutschlands Kryptogamen Flora,[7] without reference to teleutospores.

The validity of uredosporic names depends at present upon the wording of Rule 49 *bis,* which says in a rather ambiguous and illogical way that a name to be accepted must apply to the "perfect states," that is, the one "which ends in the teleutospore or its equivalent." The Rule has recently been subjected to analysis and interpretation by Faull,[8] who reaches the conclusion that, excluding the (one) example appended to the Rule, "one might very well interpret the 'state' indicated as referring to the entire diploid state, a state in the rusts that produces both uredospores and teliospores." This appears to the writer convincing and acceptable.

Returning to the name for the white-pine blister rust and recognizing Uredo names as in good standing in priority, the correct designation should be *Cronartium ribicola* (Lasch) Dietr. The name was first correctly established by Lasch under the genus Uredo and was subsequently transferred

[7] Rabenhorst, L. Deutschlands Kryptogamen Flora. Erster Band. Pilze. 613 pp. Eduard Kummer, Leipzig. 1844. (p. 579).

[8] Faull, J. H. Taxonomy and distribution of the genus Milesia. Contrib. Arnold Arboretum Harvard University, no. 2. 138 pp. Jamaica Plain, Mass. 1932. (pp. 9–11).

to the genus Cronartium by Dietrich, all in accordance with the accepted rules of nomenclature. This presentation of the subject has been read by Perley Spaulding, who states in his letter of March 8, 1933, that "I am disposed to accept your decision in the matter."—J. C. ARTHUR, Purdue University, La Fayette, Indiana.

Some Promising Fungicides.—At the Arlington Farm, Rosslyn, Virginia, station of the Bureau of Plant Industry, some promising spray materials have been developed that may be of value in the control of plant diseases and for which patent applications have been filed under the provisions of the Act of March 3, 1883, (22 Stat. 625). Because copper compounds appear to be effective in the control of fungous parasites of plants, the experiments have been principally concerned with the improvement of Bordeaux mixture and with the use of very insoluble copper compounds.

These copper compounds, when ground finely enough to pass through a 300-mesh screen or to the standard of Portland cement and mixed with milk of lime, form suspensions that lack the spreading and sticking qualities of Bordeaux mixture. To supply these qualities, an adjuvant, made by combining lime with bentonite, a natural clay-like material forming a gel with water, was developed. The gel-forming property of bentonite is not useful in the spraying of plants, since the material in this form is easily washed off the sprayed plants; but, when lime, slaked or hydrated, is added to a true bentonite, a flocculent precipitate is formed that has excellent spreading and adhering properties and furnishes a highly absorptive surface for the insoluble materials.

The copper compounds that show promise, when finely ground and combined with bentonite flocculated by lime, are: copper oxide, copper silicate, and copper phosphate. These materials are not, at the moment, manufactured for wide-scale commercial use but for experimental purposes can be bought in small quantities from the chemical-laboratory supply houses. A chemical firm has manufactured these materials for our experimental work at a considerable reduction in price over catalogue figures, and we feel sure that these compounds can be made available at very reasonable figures, should they develop into successful orchard sprays.

In a limited way we have used the above materials on Hiley peach and on York and Grimes apples. On peach, copper oxide and copper silicate controlled scab and brown rot quite well without causing injury to the fruit, foliage, or wood. Copper phosphate, although not so injurious as Bordeaux mixture, caused enough injury to peach to make its usefulness on this host doubtful. On apple copper oxide, copper silicate, and copper phosphate were not injurious in any way. Since there was practically no scab in the apple orchard no data on scab control were obtained.

In repeated laboratory tests these fungicides were toxic to the spores of *Glomerella cingulata* (Atk.) S. and S. (bitter rot of apple), *Sclerotinia fructicola* (Wint.) Rehm (peach brown rot), *Cladosporium carpophilum* Thüm. (peach scab), *Venturia inaequalis* (Cke.) Aderh. (apple scab), and *C. effusum* (Wint.) Dem. (pecan scab). The materials exerted their toxicity under the weathering conditions of the orchard environment for at least 10 days and in the case of copper phosphate as long as 21 days. This was determined by placing spores of the test fungi on the slides that had been hung among the leaves of the host and sprayed at the time the host was sprayed. A group of sprayed slides was brought into the laboratory each day and tests were run with standardized spore suspensions. In this manner the longevity of the toxic and inhibitory effect of the fungicides was determined. Bordeaux mixture was always used in comparison and usually other standard materials, such as zinc-lime, lime-sulphur solution, wettable sulphur, and proprietary sulphurs.

The liquid sprays that we used had this general formula:

Copper silicate, copper oxide, or copper phosphate (as the case may be)	2.0 lb.
Hydrated lime or equivalent CaO	4.0 "
Bentonite (one showing a high degree of water-absorbing capacity)	2.0 "
Insecticide (arsenate of lead, etc.)	1.0 "
Water	50.0 gal.

If hydrated lime is used, all the materials may be weighed together, thoroughly mixed, and added to the tank of water in a dry condition. If stone lime is used, this material must be slaked separately and added to the tank, first, in the required amount of water. Then the other ingredients, mixed together dry, may be added. In both cases the tank agitator should be in motion throughout the mixing.

We have found that a dust with good physical properties may be prepared by adding 2 lb. of the copper compound to 4 lb. of hydrated lime and 4 lb. of bentonite. To this may be added 1 lb. of an insecticide such as lead arsenate.—M. C. GOLDSWORTHY and E. L. GREEN, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Mosaic Diseases Affecting Different Legumes in Relation to Beans and Peas.—The viruses causing the mosaic diseases of white sweet clover (*Melilotus alba*), yellow sweet clover (*M. officinalis*), white clover (*Trifolium repens*), alfalfa (*Medicago sativum*), pea (*Pisum sativum*), and sweet pea (*Lathyrus odoratus*) are all transmissible to beans (*Phaseolus vulgaris*), while the virus producing the mosaic disease of red clover (*T.*

pratense) is not. Although a careful examination of the symptoms produced on beans by the viruses from these legumes reveals specific differences, the viruses from white clover and white sweet clover when inoculated into beans produce symptoms, some of which resemble the typical mottled and chlorotic condition of the common bean mosaic.

It is entirely probable that there may be present in the several legumes various other mosaic viruses that may react differently from those that are herein reported, notwithstanding the production of symptomological similarity.

The infection produced by all of the legume-mosaic viruses on beans is systemic with the exception of that of alfalfa, which produces only local lesions. The white-clover-mosaic virus, besides being systemic, may sometimes produce local lesions.

It has not been definitely proved that the viroses of the several legumes (white sweet clover, white clover, alfalfa, pea, and sweet pea) are seed-borne, but preliminary evidence indicates that the mosaics of pea and white sweet clover may be carried in the seed in small percentages. The virus of none of the legume mosaics, except bean mosaic, has thus far been proved to be transmitted through bean seed.

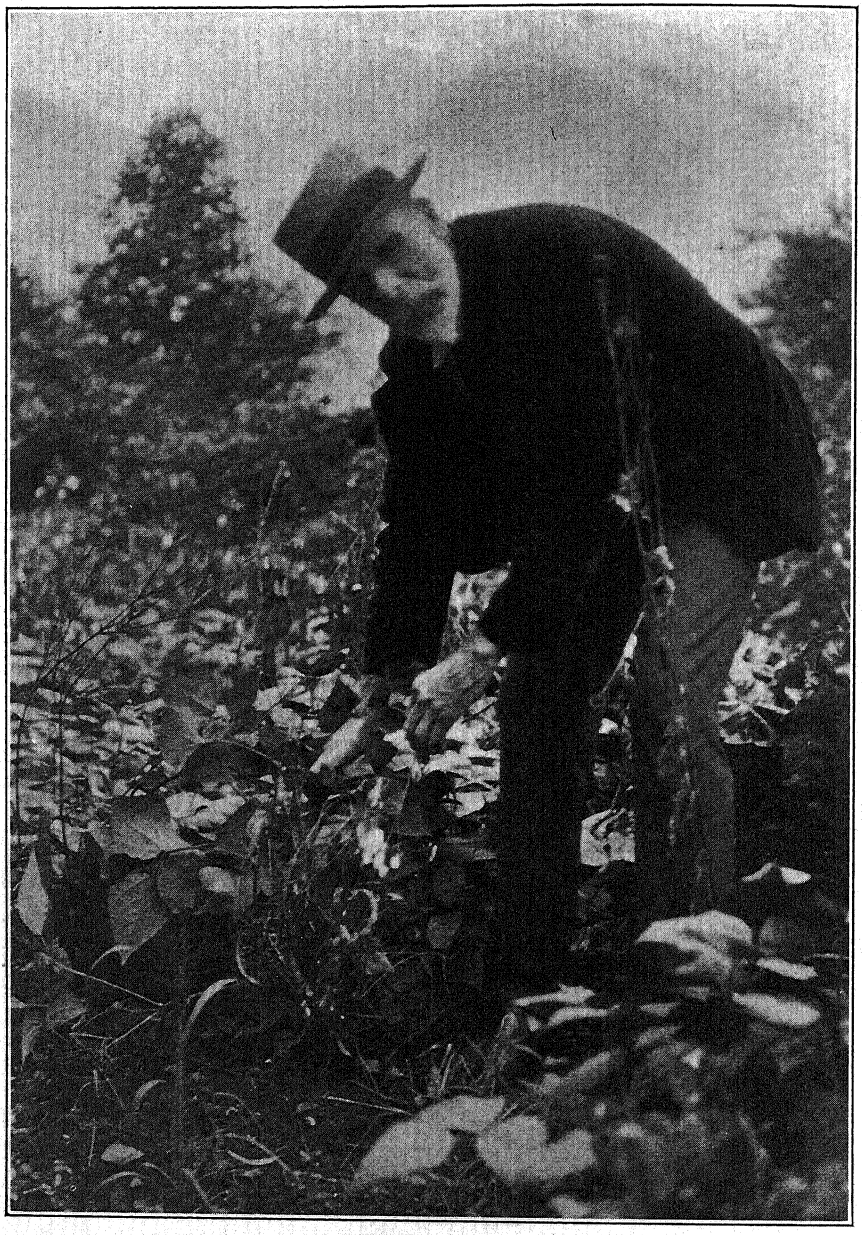
Most bean varieties are susceptible to the viruses of the legumes mentioned. The alfalfa-mosaic virus infected 24 out of 28 varieties tested, and that of white sweet clover 21 out of 22. The virus of white-clover mosaic produced typical symptoms on 24 out of 26 varieties. Thirty-two varieties of beans were tested with the mosaic virus of pea and 31 were susceptible. In the latter case the inoculated plants of many of the very susceptible varieties died.

The effect of the virus of the various legumes on bean varieties that are resistant to their own mosaic, differs, depending on the virus used. The 3 varieties of beans resistant to bean mosaic (Robust, a strain of Great Northern, and a Stringless Green Refugee selection) also are resistant to the alfalfa mosaic. The mosaic viruses of white sweet clover and white clover infect only the Stringless Green Refugee selection, while the virus of pea mosaic infects both this selection and the Robust variety. Only the strain of Great Northern appears to be resistant to all of the viruses used.

In addition to these virus diseases, a mosaic virus (designated as No. III) was isolated from a bean hybrid, whose extract reacts similarly to the legume-mosaic viruses, especially with respect to the susceptibility of certain bean varieties. It differs, however, from the common bean mosaic. The virus is systemic and does not appear to be seed-borne. Twenty-six bean varieties were inoculated with this virus and 24 were susceptible. Of the three varieties resistant to the common bean-mosaic virus, No. III was infectious only to the Stringless Green Refugee selection.

Peas and sweet peas are affected differently from beans when inoculated with the viruses of the various legume mosaics. The red-clover-mosaic virus, inoculated on the pea, produced symptoms somewhat similar to pea mosaic but gave negative results when inoculated on the few varieties of sweet pea tested. Peas and sweet peas were not infected by the alfalfa- and bean-mosaic viruses. The mosaic viruses of white sweet clover and white clover produce destructive symptoms on peas and sweet peas very unlike pea mosaic. The symptoms resemble a streak, in some cases causing death to the sweet pea. The vascular bundles of infected pea and sweet pea roots and stems are discolored and fungi are readily isolated, but in no case have they been proved pathogenic. It is likely that infected plants become weakened by the virus and that saprophytic fungi enter the root system, producing a vascular discoloration.

It is possible that both beans and peas may become infected from the various legume-mosaic viruses in the field as a result of aphid transmission. —W. J. ZAUMEYER and B. L. WADE, Division of Horticultural Crops and Diseases, U. S. Department of Agriculture.



DR. THAXTER ON HIS 70TH BIRTHDAY IN HIS GARDEN AT KITTERY POINT, MAINE, WHERE IN LATER YEARS HE STILL CONTINUED HIS INVESTIGATIONS OF PLANT DISEASES.

PHYTOPATHOLOGY

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ROLAND THAXTER (1858–1932), HIS INFLUENCE ON PLANT PATHOLOGY

WM. H. WESTON, JR.

Although known primarily for his work in mycology, none the less Dr. Thaxter exerted a distinct influence on plant pathology, not only directly through his own brief but outstanding work in this field, but also indirectly, through the inspiring example of the high standards of accuracy and completeness he maintained throughout all his investigations, through the aid in difficult mycological problems he gave to the many perplexed pathologists who consulted him, and through the training and ideals he transmitted to students who have since become important factors in phytopathology.

He was born on August 28, 1858, and naturally during the period of his school and college training he received little or none of the specialized and intensive instruction in plant pathology which is offered in the famous phytopathological laboratories of the present. It must be remembered that he entered Harvard from the Joshua Kendall School in Cambridge in 1878 only a few years after Asa Gray, with progressive foresight, had secured the appointment of G. L. Goodale in Plant Physiology and W. G. Farlow in Cryptogamic Botany. Consequently Dr. Thaxter's college course, from which he received in 1882 his A.B. degree, Magna Cum Laude, with honorable mention in Natural History and English Composition, gave him a sound background in botany and zoölogy rather than any specialized training in plant pathology. Indeed, it was not until 1884 that he began to concentrate in botany, especially mycology, when, after a year in the Medical School, he started graduate work with Dr. Farlow under two years' tenure of the Harris Fellowship.

His first mycologic paper, "On Certain Cultures of Gymnosporangium with Notes on their Roesteliae,"[1] published in 1887, was of pathologic as well as mycologic significance, since by culture studies and cross inoculations he demonstrated the relation of several species of Gymnosporangium to various Roesteliae of the vicinity. Realizing the difficulties involved, the plants of various species of Crataegus, Pyrus, and Amelanchier, potted in April before the leaves developed, had been kept in four separate rooms in Boston some miles from any cedar trees to avoid any possibility of outside

[1] For a list of Dr. Thaxter's publications see Weston, Wm. H. Roland Thaxter. Mycologia 25: 69–89. 1933. (Publications, pp. 86–89).

infection, and to these from the seven common local species of Gymnosporangium, similarly forced to an early production of telial columns, cross inoculations were made which definitely established the connection with the roestelial stages in five of the seven cases. Moreover, appreciating the possibility that sexuality and fertilization might be involved in the development of aecidia, he attempted to fertilize female organs that might be present by placing the spermatia on the under side of the leaves of the Rosaceous hosts, and although his results were not conclusive, it should be noted that he foresaw this possibility which only recently has been demonstrated as an actuality in other rusts. It should be noted also that in this connection he pointed out the possible rôle of flies in achieving such fertilization in nature, a shrewd suggestion probably derived from his thorough familiarity with the Diptera, for even at this time he had an unusual knowledge of insects and had published several entomological papers.

With characteristic tenacity he did not drop this investigation when he went on into other fields, but even while working on his thesis, "The Entomophthoreae of the United States," for which he received his Ph.D. in Natural History in 1888, he continued his study of cultures of Gymnosporangium, using a greenhouse under more carefully controlled conditions than had been possible previously. Although his procedure would now be considered heretical, as he did not use a series of plants as controls, none the less his precautions in forcing both the Gymnosporangium and the hosts so that the inoculations were made in the greenhouse a month or so before any infection occurred in nature, gave security to his results and enabled him in this paper of 1889 to corroborate his previous work, to secure in addition the roestelia of *Gymnosporangium globosum,* and by careful comparison with authentic herbarium material to establish with certainty the identity of the aecial stages which he had developed. These two papers were a worthy continuation of Farlow's "Gymnosporangia or Cedar Apples of the United States," ("cider apples," according to one biographer!) of 1880, and "Species of Gymnosporangium and Chrysomyxa" of 1885, and furnished convincing answer to the hope expressed by Peck in the Botanical Gazette of 1881 for a continuance of such investigation "until . . . the supposed connection between the Gymnosporangia and Roesteliae shall be shown to be either real or imaginary."[2]

In 1888, after receiving coincidentally both M.A. and Ph.D. degrees from Harvard, he went to his first position, that of Mycologist at the Connecticut Agricultural Experiment Station, which, as the 1907 year book of his class of 1882 takes pains to point out, "although located in New Haven, has no connection with Yale." His annual reports and other publications

[2] Peck, C. H. [Review of] The Gymnosporangia or cedar-apples of the United States, by W. G. Farlow. Bot. Gaz. 6: 209–210. 1881.

during the three years he worked in this capacity give convincing evidence in their "miscellaneous notes," of his unusual knowledge of fungi and his wide-ranging activities as a mycologist, and, in their detailed reports on important diseases, of his effectiveness as a plant pathologist. His work on the diseases of onions and particularly his reports on onion smut were valuable contributions to plant pathology, covering thoroughly the historical background and the pertinent literature, furnishing important information on the distribution, dissemination, and effects of the disease, working out in careful detail the structure, development, and methods of infection of the causal fungus, and giving practical, simple, and inexpensive methods of prevention and control based on careful experiments. Although decidedly mycological in point of view, the work is distinctly in accord with the traditions of present phytopathological procedure, comprising convincing demonstrations and illustrations of treated and untreated rows in experimental plots, and making effective use of information secured by means of questionnaires sent to persons and firms interested in onion cultivation throughout the State. Additional notes on the mildew, the Vermicularia, and the Macrosporiums of onion, as well as lists of fungi parasitic on the genus Allium, help to add to the completeness and thoroughness of this pioneer report, which still stands as significant in its field.

However, it is perhaps his study of potato scab that is the best known of his phytopathological work while at the Connecticut Station. His investigation of this disease, his thoroughgoing discussion of its history and of the theories as to its origin, his careful and convincing mycologic work in separating the secondary fungi, isolating the causal organism and proving its pathogenicity, make this one of the most interesting and important of his plant pathological papers. The work on potato scab he continued during a second season, confirming by experiments his previous conclusions, answering conclusively the doubt of other investigators as to his results, and in the preventive measures that he had found advantageous, emphasizing especially the importance of using seed free from scab.

That this young investigator, primarily a mycologist in training and inclination, with ready adaptability could turn all the resources of his training, his knowledge, and his natural ingenuity to the practical problems of the control of plant diseases, was well demonstrated also in his work on the leaf spot of quince, which at that time had become so serious in Connecticut that some orchards were of necessity being abandoned. Enlisting the cooperation of some of the growers, Dr. Thaxter made careful experiments in spraying with fungicides through parts of their orchards, leaving other parts untreated as controls. For this work he devised a spraying outfit, consisting of a copper wash boiler with a hand force pump and a Vermorel nozzle, and this apparatus, although costing only a little over eight dollars,

and crude in comparison to the improved modern types, proved superior to the Eureka sprayer owned by the station, and is of interest to us now as one of the earliest outfits used in the pioneer days of plant-disease control by fungicides in this country. The work on controlling leaf spot of quince, which he carried on through the seasons of 1890 and 1891, demonstrated conclusively that from a single treated row could be secured a net income one hundred times as great as that from an untreated row, and on the practical basis of such impressive balance of yield over expense, went far toward converting even the most hard-headed and unprogressive growers of that time to the then "new-fangled" practice of preventive spraying. Although the major part of his investigation was concerned with quince leaf spot, he tried out various fungicides on black rot of grapes as well, and, by interesting progressive farmers and growers in coöperative spraying experiments, secured very satisfactory results with leaf spots of plums and cherries, and with other diseases such as late blight of potato. His reports on spraying and the use of fungicides in plant-disease control took up in detail the suitability of various fungicides, the best methods and times of application, the requisite features of the apparatus, the costs involved and the yields secured.

While at the Connecticut Station also, he described *Phytophthora phaseoli*, a new species on Lima Bean, from the pathologic point of view discussing its destruction, distribution, method of spread, and effect on the host, and from the mycologic point of view examining its distinguishing characteristics and establishing it as a new species. It is a tribute to his mycologic knowledge and discernment that this species had retained its specific rank through the recent critical physiologic, biometric, and cultural reinvestigations in which so many species have been relegated to synonymy.

Shorter papers, both the continuation of his work on Gymnosporangium in "The Connecticut species of Gymnosporangium (cedar apples)" and his study of the fungus in violet roots, *Thielavia basicola*, although short notes, show the same characteristic care and thoroughness in investigation.

He was mycologist at New Haven for only three years (1888–1891) but in this time he contributed definitely to the development of plant pathology. His work showed a sound mycological background, a wide knowledge of the pertinent literature, a flair for convincing experimentation, a ready ingenuity and adaptability coupled with conservative persistence and exacting accuracy. As Clinton,[3] in his vice-presidential address on "Botany in Relation to Agriculture" has pointed out, Dr. Thaxter advanced plant pathology materially by important work on the artificial culture of pathogenic fungi and by pioneer contributions to plant-disease control through preventive spraying for quince leaf blight, seed selection from potato scab,

[3] Clinton, G. P. Botany in relation to agriculture. Science n.s. 43: 1–13. 1916.

and sulphur treatment for onion smut. Yet, through all his earnest devotion to his work he retained his characteristically dry sense of humor, for in his copy of the report of an annual agricultural convention in 1888, in the announcement "The quartet again favored the audience with a song, after which the Chairman introduced as the lecturer of the afternoon, Dr. Roland Thaxter" he starred the word "song" and at the bottom of the page added in his precise handwriting, "*Entitled "Oh Spare me! Spare me! Free me from this pain!"

Moreover, during these years, although carrying on pathological work that would have overwhelmed many men, he still found time to continue purely mycological investigations which led to descriptions of interesting new genera and species of the Imperfecti in two papers on "Certain new or peculiar North American Hyphomycetes" in 1891, and through successful culture of a Chondromyces parasitic on lichens around New Haven revealed the true nature of this organism on which as a basis he later established the Myxobacteriaceae as a new order.

In 1891, the period of Dr. Thaxter's direct contributions to phytopathology ended and that of his indirect influence as a teacher began, for he was called back to Harvard to be assistant professor associated with Dr. Farlow. Active in research and in teaching, he was given ever-increasing responsibility, as Dr. Farlow in 1896 gave up the instruction of undergraduates; and from 1901, when he became full professor, he carried the full burden of Cryptogamic Botany until after Dr. Farlow's death in 1919 he became Professor Emeritus and honorary Curator of the Farlow Library and Herbarium, voluntarily retiring to devote his remaining years to his investigation of the Laboulbeniales.

During these years nearly a thousand students attended courses under Dr. Thaxter or worked with him as graduate students for higher degrees. Although these courses, the more elementary in Cryptogamic Botany and the more advanced ones devoted to the fungi, algae, and bryophytes, gave no specific intensive training in plant pathology as such, none the less they inculcated ideals of exacting accuracy and painstaking thoroughness, furnished an indispensable background, and gave much directly valuable training and information on culture methods, life histories, and measures of prevention or control. It is true that of the men especially trained for their life work by studying as graduate students under Dr. Thaxter, only a relatively small proportion devoted themselves to plant pathology as a profession, yet from this number between 20 and 30 have since been influential, ranging from some of his earlier students such as J. B. Rorer and J. R. Johnston, whose names are known in tropical plant pathology, and G. P. Clinton, H. P. Barss, H. S. Jackson, and J. H. Faull, active in plant pathol-

ogy in the United States and in Canada, through R. H. Colley, W. A. McCubbin, and O. F. Burger of more recent years, down to Charles Drechsler and F. C. Meier among the last of the men trained by him before he relinquished teaching in 1919.

His indirect influence on plant pathology, however, was not exerted solely through his training such able men as these. His extensive correspondence furnishes ample evidence of the invaluable assistance he gave the many pathologists who, recognizing his extraordinary knowledge of the fungi, turned to him for help when in doubt over the identity or behavior of obscure or perplexing forms. Moreover, the high standards of accuracy, thoroughness, and completeness which he maintained throughout his own work, and upon which he insisted in the work of his students, unquestionably exerted a beneficial influence not only on mycology but on plant pathology as well.

In his later years Dr. Thaxter seems to have gained the reputation of being rather critical of plant pathology. It should be remembered, however, that he was ever ready to commend and esteem work of high quality whether it was done in the field of plant pathology and applied mycology or in the more restricted field of pure mycology. He had, it is true, a justifiable scorn for incomplete and inaccurate work, work lacking accuracy and thoroughness in investigation and experiment, deficient in familiarity with the pertinent literature, inadequate in necessary mycological background, and inferior in the quality of its presentation and illustrations. It was not that he deplored phytopathology as such, but rather that the very incompetence and complaisance with poor work which he decried seemed to him to be more prevalent in phytopathology than in the academic phases of botany. Even the famous "battle of Kittery Point," which has become a legend where mycologists and plant pathologists gather, was but an active expression of this same insistence on high standards, a natural impatience that in a campaign toward plant-disease control, involving complex biological problems, power should be vested in those without adequate training or knowledge to administer that power to its best ends. For the most part Dr. Thaxter's reputed scorn for phytopathology is the result of his dryly humorous allusions in such phrases as "squirt gun botanists" or "mycology of the stomach and the pocket book." As he himself had been a pioneer in the control of plant diseases by spraying, the irony of his phrase "squirt gun botanists" was obvious to himself as well as to plant pathologists, and when Clinton once sent him a Connecticut spraying calendar with an illustration of Dr. Thaxter's own hand-made washboiler spray outfit with the inscription "squirt-gun botanist," he enjoyed the joke keenly and hung the inscribed illustration on the wall among his curios. His allusions to "mycology of the stomach and the pocket-book" also were but

a humorous expression of his regret that suitable opportunities were so few that men with great potentialities for productive scholarship were forced into routine governmental or industrial positions. For example, when Dr. Lyman, after being the guiding spirit of so many of us who were fortunate enough to come under his stimulating influence at Dartmouth, had gone to the Federal Horticultural Board, Dr. Thaxter, in discussing possible openings with the writer, remarked with a sigh, "Of course one can always get some sort of position in Washington, and even able men like Dr. Lyman seem to like it there, I'm sure I don't know why!" He felt that it was a great loss when promising men were forced by economic conditions from the academic field of education and productive research, because it was his sincere conviction that teaching and research were the important and fundamental basis of a cultured and advancing civilization.

An outstanding scholar and investigator, an effective teacher, his nearly fifty years of devoted work exerted a significant influence far beyond his own sphere of endeavor, and his death on April 22nd, 1932, was a great loss not only to the field of mycology in which he has become internationally renowned, but to the related field of plant pathology as well.

LABORATORIES OF CRYPTOGAMIC BOTANY,
HARVARD UNIVERSITY.

A CYTOLOGICAL STUDY OF THE TELIOSPORES, PROMYCELIA, AND SPORIDIA IN PUCCINIA MALVACEARUM[1]

RUTH F. ALLEN[2]

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Puccinia malvacearum Mont., the rust of Althaea, Malva, and related genera, is the cause of a wide-spread disease and has been studied by botanists in many lands. According to Eriksson (12), the original home of the disease is either Chile, where it was found in 1852, or Australia, where its presence was recorded in 1857. It was found in Spain in 1869, in France in 1872, in England and Germany in 1873, in Belgium, Holland, Denmark, and Italy in 1874, and so on, throughout Europe.

Much of the early work on *Puccinia malvacearum* was devoted to learning its life history and mode of overwintering. Kellerman, in 1874 (21), recognized it as a short-cycle rust and traced its entrance into the host and the development of its mycelium. Plowright, in 1882 (31), followed the rust through successive telial generations and studied the formation of the promycelia. Both found that the rust overwintered as pustules, in sheltered spots.

According to Dudley (11), the rust appeared in America in 1886. Dandeno in 1907 (8), Taubenhaus in 1911 (37), and Hill in 1925 (16) studied the overwintering of *Puccinia malvacearum* in America, the latter concluding that "the immature teliospores of *Puccinia malvacearum* are able to withstand the severity of winter in Quebec (minimum temperature −35° F.) and germinate the next spring, producing infection of healthy plants."

The extensive work of Eriksson on *Puccinia malvacearum* in 1911 (12) and 1921 (13) was devoted to a proof of the mycoplasm theory, while that of Bailey, on this rust, in 1920 (2), consists of a refutation of that theory.

Robinson, in 1913 (33), studied the relations of *Puccinia malvacearum* to its host, the tissues invaded, and the effects of the fungus upon these tissues.

Sappin-Trouffy, in 1896 (35, 36), published extensive cytological studies in the Uredineae, including a brief account of *Puccinia malvacearum*. He found that the mycelial cells are uninucleate and the haustoria are variable, "*fourchus, spiralés, rameux, utriculaires, ou même pelotonnés.*" "*Le*

[1] Cooperative investigations between the California Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Acknowledgments are due the departments of Agronomy and Genetics of the University of California for courtesies extended to the writer during the progress of this investigation.

dévellopement ainsi que les phénomènes de fécondation [*i.e.*, nuclear fusion] *de la téleutospore sont les mêmes que dans les autres Puccinia.*'' The teliospores germinate without a rest period, each cell giving rise to a curved promycelium into which the fusion nucleus migrates and then divides twice. If the teliospore is immersed in water the development is abnormal, the promycelium segmenting into conidia without forming sporidia.

While there have been a number of more recent cytological investigations of this rust, most of them, including the work of Blackman and Fraser, in 1906 (4), Olive, in 1911 (30), Werth and Ludwig, in 1912 (39), Moreau, in 1914 (28), and Lindfors, in 1924 (22), are concerned primarily with the mode of origin of the binucleate condition.

Jackson, in 1931 (19), in a discussion of the evolution of rusts, cites *Puccinia malvacearum,* a microcyclic form without pycnia, as a type probably homothallic, and Ashworth, in 1931 (1), presents as evidence of homothallism experiments showing that this rust, when grown in monosporidial culture, produces normal teliospores.

In all this work on *Puccinia malvacearum* little attention has been given to the nuclear details during the formation and germination of the teliospores. As the material is particularly favorable for such a study, the following account is presented.

MATERIAL AND METHODS

Teliospores of *Puccinia malvacearum* can germinate without a rest period, but, if conditions for germination do not occur, they retain viability for a considerable length of time. In July, at Berkeley, California, after a month or two without rain, the teliospores germinate with great rapidity and in great abundance. Hollyhock leaves, bearing infections, were washed and placed in Petri dishes lined with wet paper and the sori were fixed at half-hour intervals from 1½ to 5 hours thereafter.

For the study of the development of the teliospore, a young potted mallow plant was inoculated. A pad of wet paper was placed in a watch crystal; a rusted hollyhock leaf was placed on it and secured in position by rubber bands. A glass cylinder lined with wet paper was set over the plant and the watch crystal inverted and placed on it as a lid. A layer of wet paper was folded down about the top and secured by a rubber band. This placed the germinating teliospores directly over the plant in a small damp chamber. The plant was then heavily watered and placed under a greenhouse bench for 48 hours. Infections developed in abundance and material was fixed 10 to 12 days after setting up the inoculation, when the new crop of teliospores was developing.

The material was fixed in Flemming's medium and weak solutions and in chrom-acetic-urea. It was washed, dehydrated, imbedded in paraffin,

and sectioned. Safranin and methylene blue were used to stain the young teliospores. Iron-haematoxylin was found advantageous in studying the mature and germinating teliospores, as it left the heavy teliospore wall unstained and so permitted the study of its contents.

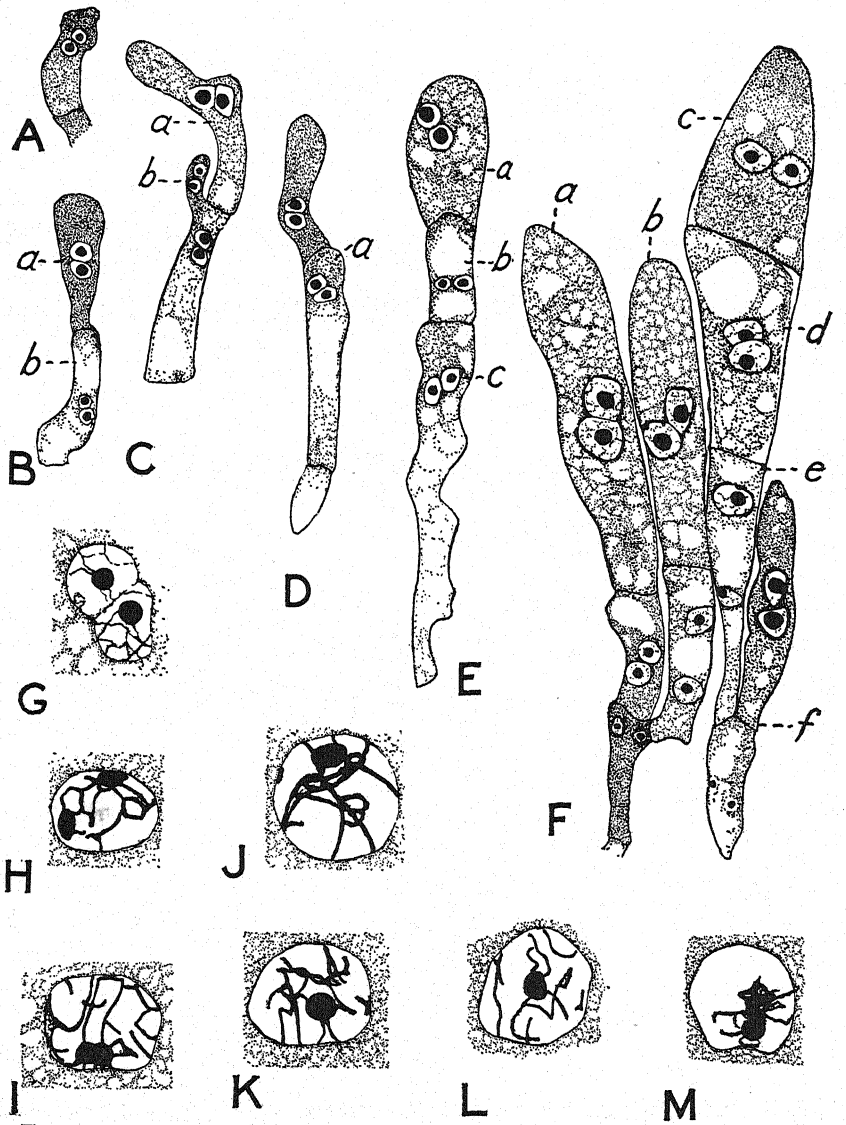


FIG. 1. *Puccinia malvacearum.* A to F, stages in the development of the teliospores; G to M, nuclear fusion and prophase of heterotypic division. A to E, ×1,020; F, ×1,400; G to M, ×2,250.

INVESTIGATIONS

Teliospores arise from binucleate cells. In figure 1, A, is drawn a young binucleate cell at a stage just before the initiation of spore formation. Nuclei and cytoplasm are concentrating at the distal end of the cell. It divides (Fig. 1, B) the terminal cell, *a*, receiving a pair of daughter nuclei and the bulk of the cytoplasm. This is the teliospore initial. The basal cell (Fig. 1, B, *b*) grows larger, its nuclei move to the apex of the cell (Fig. 1, D, *a*), divide, and a branch pushes out, which receives a pair of the daughter nuclei (Fig. 1, C, *b*). This is the beginning of a second teliospore. This branch soon becomes isolated from the parent by a septum (Fig. 1, F, *f*). If the sorus is well nourished this may be repeated, the same basal cell giving rise successively to 3 and even 4 teliospores. At this stage the teliospore initial is not infrequently bent (Fig. 1, C, *a*), being constrained into irregular form by the pressure of adjoining cells.

The teliospore initial grows and divides, the terminal cell (Fig. 1, E, *a*) becoming the spore and the second cell (Fig. 1, E, *b*) its stalk. The terminal cell becomes larger (Fig. 1, F, *a*, *b*) and divides to form the 2 cells of the teliospore (Fig. 1, F, *c*, *d*), while the stalk cell (Fig. 1, F, *e*) grows long and slender.

Soon after the last division the pair of nuclei in each cell of the teliospore fuse. At the time when the 2 nuclei become flattened against each other (Fig. 1, G) each contains a large spherical nucleolus and a number of delicate chromatin strands.

The nuclear membrane between the 2 now breaks down (Fig. 1, H) and the outline rounds out into an ellipsoid. The chromatin strands are fewer in number than the sum of those of the 2 component nuclei and are heavier. This may be due to a pairing of the strands; in fact, occasional hints of this can be seen, although in so minute a nucleus it would be difficult to prove. The nucleoles are still some distance apart. A slightly later stage is drawn in figure 1, I. The nucleus has grown, the chromatin strands are more uniform in diameter, and the nucleoles are fusing. In figure 1, J, the nucleolar fusion is complete. At no time has the nucleole been seen disconnected from the chromatin strands. It may be that here, as is maintained for higher plants, the nucleole occurs at a definite locus in a chromosome. If so, the nucleolar fusion here would be further evidence of chromosomal pairing at this stage. The figures G to J are drawn at the same magnification (×2,250). A comparison of the drawings shows that the volume of the nucleus has increased rapidly during the fusion.

The sequence of the stages represented in figure 1, K, L, and M, is not certain, but L and M probably represent later stages. Figure 1, M, resembles closely the synizesis of higher plants.

Figure 2, A, shows a teliospore (drawn at lower magnification than the preceding) at the time when nuclear fusion is in progress. In the lower cell the 2 nuclei have just combined; in the upper, fusion is complete. In figure 2, B, is drawn the mature teliospore with its firm wall, dense cytoplasm, and completely fused nuclei.

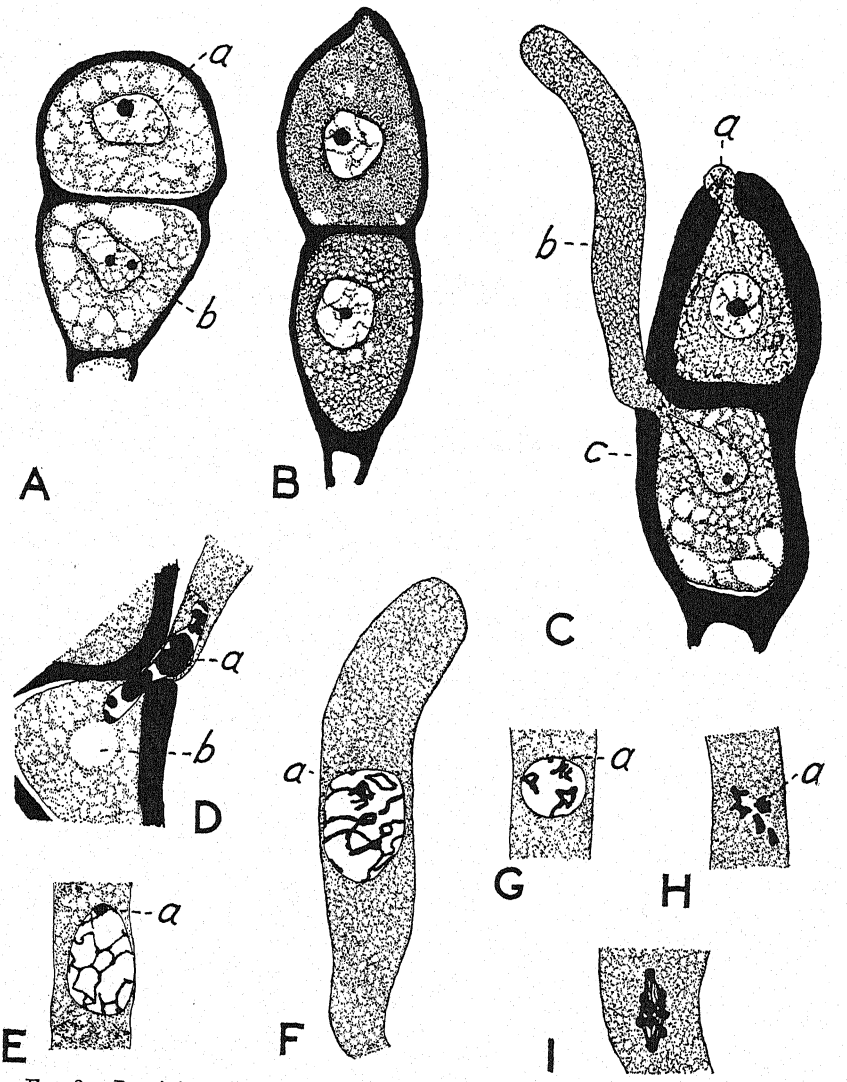


FIG. 2. *Puccinia malvacearum*. A, maturing, B, mature, and C, germinating teliospore; D, transit of nucleus to promycelium; E to H, prophases of heterotypic division; I, early anaphase. A to C, ×1,400; D to I, ×2,250.

After $2\frac{1}{2}$ hours in a damp chamber the teliosori are covered with young promycelia. Figure 2, C, shows an early stage. The distal cell of the spore is just beginning to push out at *a* and the proximal cell has formed a hypha, *b*, as long as the spore. It contains only cytoplasm, however, for the nucleus, *c*, is still in the spore, although it is elongating in the direction of the germ pore.

The transit of the nucleus through the germ pore has been seen 2 or 3 times and in each case the condition in the nucleus is as represented in figure 2, D, *a* (drawn at high magnification). This concentration of the chromatin into lumps may be due, at least in part, to bad fixation, although it is not clear why this stage should be specially vulnerable when the stages just before and after it are well fixed. The exit of the nucleus leaves a vacuole, *b*, in the parent cell.

Immediately after its exit into the young promycelium the nucleus expands again (Fig. 2, E) and the chromatin loosens up into delicate strands. As the prophases of the first division progress, the chromatin threads shorten and thicken (Fig. 2, F) until finally the short, thick chromosomes can be recognized (Fig. 2, G). During this period a small granule (Fig. 2, E, *a;* F, *a;* G, *a*) appears at the distal end of the nucleus. It is probably not the nucleole. It may be a centrosome such as has been described for other fungi, but proof of this is lacking.

Next, the nuclear membrane disappears and the short, lumpy little chromosomes (Fig. 2, H) are scattered loose in the cytoplasm. Each consists of at least 2 parts and the one at figure 2, H, *a*, could easily be interpreted as consisting of 4, corresponding to the "tetrads" in the heterotypic division of higher plants. An attempt has been made in this and subsequent stages to determine the chromosome number. The minuteness of the division figures makes this difficult, but when the figures are clearest the number appears to be 5.

The spindle forms with its main axis parallel to the length of the hypha. Figure 2, I, shows an early anaphase with the dense little chromosomes crowded upon it. Figure 3, A, shows another anaphase with a broader spindle on which the chromosomes are better disposed for counting. There seem to be 5 in each group of daughter chromosomes. The little dark-stained spot at each pole again suggests a centrosome but, of course, may be only the denser meeting point of many fibers. Some 40 or 50 such division figures have been seen and, whatever it is, this dark-stained polar spot is regularly present.

In figure 3, B, is represented a typical late anaphase. The chromosomes have not moved back to the pole at an even rate: 1 pair, *a*, is still at the equator; another, *b, b*, is half way to the poles; and the rest, *c, c* (probably 3, if one may judge by the shape of the mass), are at the poles. The line

from pole to pole, composed presumably of fibers, is regularly present at this stage.

The second division follows quickly upon the first; in fact, a resting stage between the two is not readily found. Figure 3, C, shows the 2-cell promycelium, each cell with a nucleus in prophase. As these nuclei are even smaller than those of the first division, little detail can be made out.

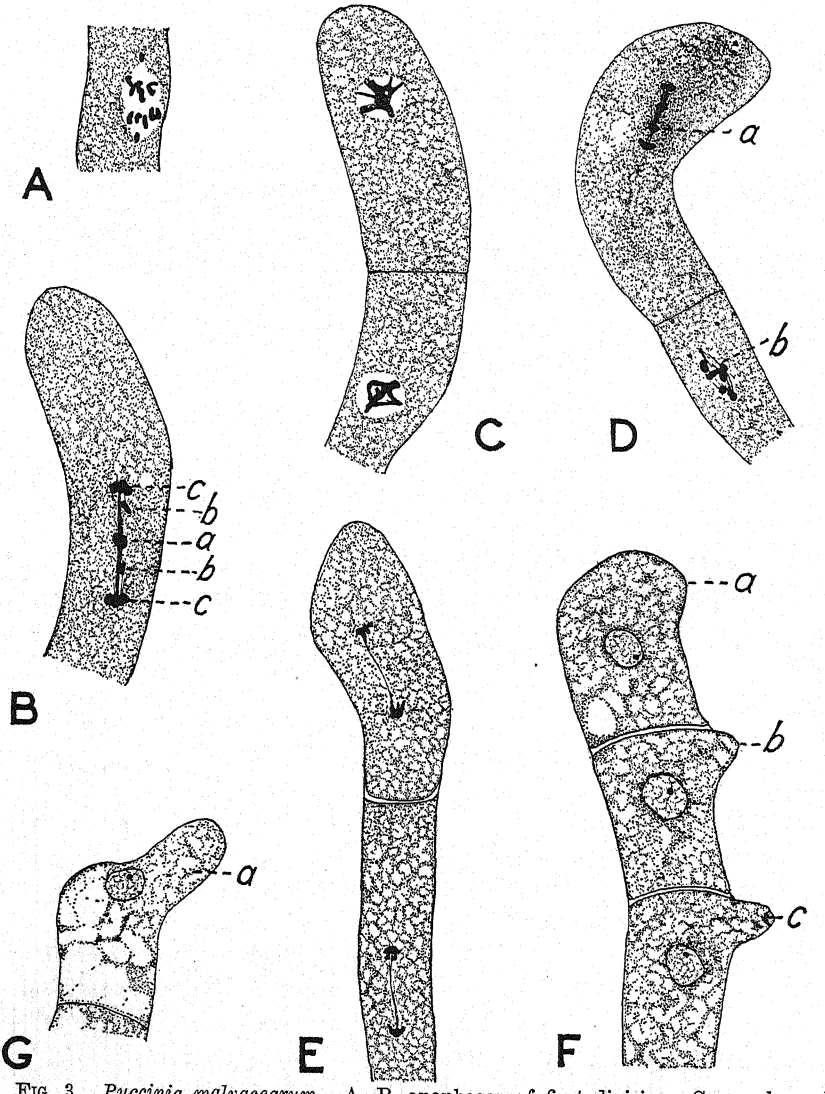


FIG. 3. *Puccinia malvacearum.* A, B, anaphases of first division; C, prophase, D, anaphases, and E, telophases of second division; F, G, beginning of sporidium formation. A to G, × 2,250.

In figure 3, D, the division has made unequal progress in the 2 cells; the lower cell at *b* is in early anaphase; in the upper, division is more advanced. In figure 3, E, are late telophases. The second nuclear division is quickly followed by cell division as the nuclei enter the resting condition.

The upper part of a 4-cell promycelium is shown in figure 3, F. Already, a blunt projection has pushed out at the apex of each cell (Fig. 3, F, *a, b, c*) to form the sporidia. Figure 3, G, shows the apical cell of a

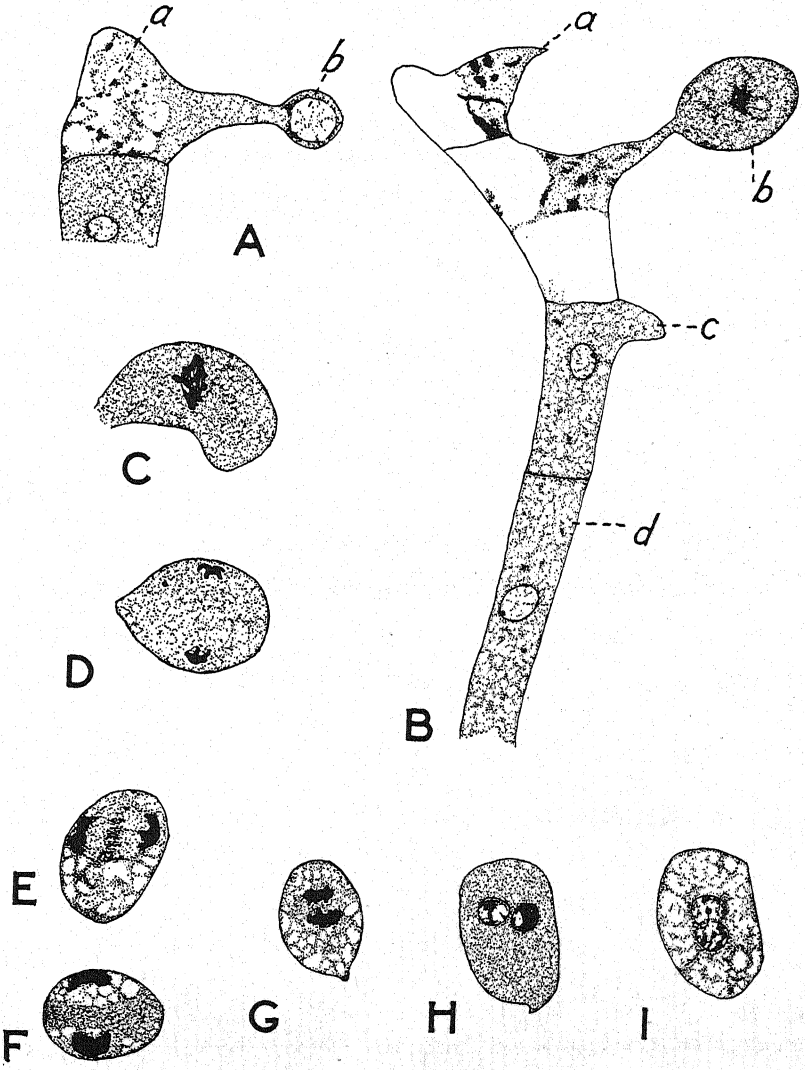


FIG. 4. *Puccinia malvacearum.* A, young sporidium; B, mature promycelium; C, metaphase and D to G, telophases of nuclear division in sporidium; H, I, mature binucleate sporidia. A to I, × 2,250.

promycelium somewhat further advanced in the process; a broad beak has formed at *a*.

As the sporidial beak elongates, a terminal swelling forms into which the nucleus moves (Fig. 4, A, *b*) leaving the parent cell, *a*, enucleate and with vacuolate cytoplasm.

Sporidia form in sequence on the promycelial cells, the apical cell commonly taking the lead. In figure 4, B, the terminal cell, *a*, has discharged its sporidium, the second cell, *b*, bears a nearly mature sporidium, the third, *c*, is just pushing out, and the fourth, *d*, shows no indication of spore formation.

The nucleus in the young sporidium in figure 4, A, *b*, is in the resting condition; that of the larger sporidium in figure 4, B, *b*, is in prophase of division. Figure 4, C, shows a metaphase of division with the spindle placed transversely in the sporidium. The chromosome number is probably 5. Ordinarily the sporidium is discharged before the division is completed. Later stages of division are abundant in newly discharged sporidia. Figure 4, D, E, F, and G, shows steps in the telophase and reconstruction of the daughter nuclei. Sometimes the plane of division is transverse, sometimes longitudinal. In figure 4, H and I, the division is complete and the binucleate sporidium is ready to germinate.

DISCUSSION

A number of cytologists have attempted to study nuclear divisions in the rusts, but the nuclei are so small that little detail can be made out with certainty. The earlier work (Rosen in 1892 (34), Poirault and Raciborski in 1895 (32), Sappin-Trouffy in 1896 (35, 36), and Juel in 1898 (20)) gave scant results. For some of the workers, even the formation of a spindle in connection with division was a debatable question. What could be seen was painstakingly recorded, but poor fixation had altered or destroyed many details. With later improvements in the technique, however, it has become recognized that nuclear divisions in the Uredineae are much like those of higher plants.

Statements concerning the chromosome number in rusts are usually hesitant and qualified; it is difficult to be certain. Poirault and Raciborski, in 1895 (32), found but 1 chromosome in a nucleus in *Peridermium pini acicolum* Link, *Puccinia liliacearum* Duby., *Aecidium leucospermum* D. C., and *Coleosporium euphrasiae* (Schum.) Wint. They state that "*. . . chaque noyau ne contient qu'un seul chromosome, mais c'est une caryokinése typique.*" Sappin-Trouffy in 1896 (35, 36), reviewed later by Tischler (38), studied representatives of the genera Endophyllum, Cronartium, Melampsora, Thecopsora, Gymnosporangium, Uromyces, Puccinia, Phragmidium, Triphragmidium, and Coleosporium and found the chromo-

some number to be uniformly 2. Juel, in 1898 (20), studied *Coleosporium campanulae* (Pers.) Lév. and found no differentiation of the chromatin into chromosomes during division. Maire, in 1900 (24) and in 1902 (25), found 2 chromosomes in divisions in *Puccinia liliacearum, P. bunii* D. C., and *Endophyllum sempervivi* Maire. Holden and Harper, in 1902 (18), found 6 to 10 chromosomes in *Colesporium sonchi-arvensis* Lév. Blackman, in 1904 (3), found "at least 10" chromosomes in the heterotypic division of *Gymnosporangium clavariaeforme* Rees but found that the vegetative divisions are "of such a simple nature that chromosome-formation is in complete abeyance." Christman, in 1905 (6), did not count the chromosomes but figured more than 2 in divisions in the aecium of *Caeoma speciosum* Fr. Olive, in 1908 (29), found 8 chromosomes in *Triphragmidium ulmariae* (Schum.) Link. Hoffman, in 1911 (17), studied the heterotypic division of *Endophyllum sempervivi* Lév. and found the chromosome number to be probably 8. Moreau, in 1913 and 1914 (26, 27, 28), found 2 chromosomes in *Phragmidium subcorticium* Schrank., *Coleosporium senecionis* Pers., and *C. sonchi* Pers. Colley, in 1918 (7), working on *Cronartium ribicola* Fischer, found more than 2 chromosomes and stated that "possibly 8 is the haploid number." Lindfors, in 1924 (22), found 4 chromosomes in nuclear divisions in *Puccinia arenariae* (Schum.) Wint. Dodge and Gaiser, in 1926 (10), studying *Caeoma nitens* Burrill, found the nucleus polarized in prophase and counted at least 14 loops.

The chromosome number for *Puccinia malvacearum* was not determined definitely, but the probable number is 5.

From the beginning of the work on nuclear divisions in rusts down to the present, workers have been divided on the question of the existence of a centrosome with or without polar radiations. Among the advocates of the centrosome are Juel (20), Holden and Harper (18), Blackman (3), Christman (6), Olive (29), Moreau (26), Colley (7), and Lindfors (22), but, here, as in all other questions affecting certain of the details of mitosis in the rusts, the minuteness of the nuclei and of the division figures makes it difficult to be certain.

There is a little evidence of a centrosome in *Puccinia malvacearum*. It is easy enough to find a small speck on the nuclear membrane of the resting nucleus and a tiny dark-stained area at the spindle poles during division, but this in itself does not prove the existence of a centrosome. The speck on the membrane may be merely one of the cytoplasmic food granules accidentally located there, and the dark spot at the spindle poles may be just the denser meeting point of many fibers. No polar radiations have been noted. Until the supposed centrosome can be traced through all stages of nuclear division and its behavior as a centrosome can be proved, it cannot be stated with certainty that a centrosome is present in *P. malvacearum*.

The one conspicuous object in resting nuclei of rusts is the single spherical nucleole. Students of rust cytology agree that during vegetative divisions this nucleole moves out into the cytoplasm, remains there through the metaphase, and gradually disappears during the anaphase and telophase. This has been observed and figured by Poirault and Raciborski (32), Sappin-Trouffy (36), Maire (24, 25), Christman (6), Olive (29), Dittschlag (9), Hoffman (17), Moreau (26, 28), and Fromme (14).

Quite different is the behavior of the nucleole during nuclear fusion. In *Puccinia malvacearum,* when the 2 nuclei fuse in each cell of the ripening teliospore, the 2 nucleoles, at first far apart, move closer together and then fuse. A similar nucleolar fusion in the teliospore (or in the aeciospore, in Endophyllum) has been observed in one or more rusts by Poirault and Raciborski (32), Sappin-Trouffy (36), Maire (25), Holden and Harper (18), Blackman (3), Hoffman (17), and Moreau (28). So far as can be judged, nucleolar fusion during nuclear fusion is of general occurrence in the rusts.

This nucleolar fusion is of interest in connection with the question of chromosomal pairing. Heitz (15) has shown that in a number of higher plants the nucleoles occupy definite loci on specific chromosomes. A nucleole appears first in the telophase as a ring around a thin place in a chromosome, then grows larger and rounds out. In the prophase of the next division the nucleole disappears gradually, again exposing at the same place the thin thread in the chromosome. The much-discussed "satellites" of chromosomes are merely the portions, large or small, of the chromosomes distal to the thin threads left at nucleolar loci. McClintock (23) and Burnham (5) have proved that in the reduction divisions of maize when the 2 "satellite chromosomes" pair the corresponding nucleoles are brought together.

It is perhaps a far cry from the nuclei of higher plants to those of rusts, but the regularity with which the nucleoles move together and fuse in the newly fused nucleus at once suggests that here, too, a nucleole occupies a definite locus in a chromosome and that it is the synaptic pairing of the homologous chromosomes that brings the nucleoles together.

It is an open question whether the synizesis in figure 1, M, is an artefact. If so, the chromatin at this stage is exceptionally sensitive to the fixing fluid, for figures such as this are not infrequent in otherwise well-fixed material. Moreover, such figures occur in other rusts and have been recorded by Holden and Harper (18), Blackman (3), Hoffmann (17), and Moreau (28).

The early stages of the prophase of the heterotypic division take place in the teliospore cell; the later stages wait until the nucleus has moved out into the promycelium. During transit, the nuclear content becomes so

greatly condensed that no detail of chromatin structure is visible and it is difficult to believe that the organization of the chromatin is maintained. This, too, has been observed in other rusts. Blackman (3) says that the nucleus is "very much constricted and condensed in its passage through the pore." Lindfors (22) speaks of the difficulty of making out the structure "*wegen des zusammengepressten Zustandes des Kerns.*" Although this transit takes place during division, the chromatin structure is not lost or impaired and division is resumed as soon as the nucleus rounds up again in the promycelial tube.

The 2 divisions follow in quick succession. If there is a true resting stage between the first and second divisions it is very brief. A few workers (3, 7, 18, 28) have figured it.

Binucleate sporidia appear to be fairly common in rusts. They have been observed by Poirault and Raciborski (32) in *Coleosporium euphrasiae;* by Sappin-Trouffy (36) in *Endophyllum euphorbiae sylvaticae* (D. C.) Wint. and *Puccinia malvacearum;* by Maire (24) in *Endophyllum sempervivi* Pers.; by Holden and Harper (18) in *Coleosporium sonchi-arvensis;* by Blackman (3) in *Phragmidium violaceum* Wint. and in *Gymnosporangium clavariaeforme* Rees; by Colley (7) in *Cronartium ribicola;* and by Lindfors (22) in *Puccinia arenariae.*

It has been usually taken for granted, as stated by Blackman (3, p. 329), that the nuclear division in the sporidium "appears to be entirely without significance and to be merely a precocious division of the nucleus in which the usual wall-formation is delayed" and that "the sporidia have been observed to give rise to a mycelium with single nuclei." A few (18, 32) have assumed that the binucleate sporidium grows directly into binucleate mycelium. In the case of *Puccinia malvacearum,* however, Ashworth (1) has figured binucleate sporidia and states that the early mycelium is uninucleate.

Lindfors (22) reports the unique case of *Puccinia arenariae* a leptopuccinia. When the teliospore germinates the nucleus divides twice but the promycelium remains bicellular, each binucleate cell of it giving rise to a single binucleate sporidium, which grows directly into binucleate mycelium.

Puccinia malvacearum is a rust without aecia or uredinia. It is maintained as a continuous series of telial generations unaccompanied, so far as known, by spermogonia. It is of interest, therefore, that there is no hint of degeneration in the reproductive processes. In fact, the reduction divisions here (in such details as can be seen in these tiny nuclei) appear to be as exact and efficient as the corresponding processes in higher plants.

SUMMARY

In *Puccinia malvacearum* teliospores are formed by binucleate basal cells each of which may by branching give rise to several spores in succession. Each spore initial divides into spore and stalk cell and the spore cell divides again to form the 2-cell spore.

The 2 nuclei in each cell of the spore fuse. The 2 large nucleoles in the fusion nucleus fuse and there is some evidence that the chromatin strands pair.

The long slender chromosomes, which wind about the nuclear cavity, now shorten and thicken into little lumps that are at least double and possibly quadruple. The nucleole and the nuclear membrane disappear. No sharply defined equatorial-plate stage has been noted; the chromosomes, probably 5 in number, lie scattered along the length of the spindle. The chromosomes divide and pass back to the poles, some arriving ahead of others.

A cell wall divides the promycelium into 2 cells and a second nuclear and cell division follows quickly.

Each cell pushes out a broad beak at its upper end, which swells at its tip to form the sporidium. The nucleus moves out into the young sporidium and divides, the later stages of the division taking place after the sporidium is discharged. Mature sporidia are regularly binucleate.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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A NEW SCLEROSPORA FROM NYASALAND

WILLIAM H. WESTON, JR.[1]

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Collections within recent years have shown that we may expect the discovery of interesting new species of Sclerospora, such as *S. northi*[2] and *S. noblei*,[3] on wild grasses in regions in the Eastern Hemisphere where little intensive mycological work has been done. It was a fortunate circumstance, therefore, that Dr. E. J. Butler, Director of the Imperial Mycological Institute at Kew, was called to Nyasaland, Southeast Africa, to make a survey of the diseases of tea and tobacco, because during his years of constructive mycological work in India Dr. Butler had become thoroughly familiar with the Sclerosporas, the importance and destructiveness of which he had been one of the first to emphasize. While engaged in this survey he collected *S. graminicola* Sacc. Schroet. on *Pennisetum typhoideum* at the Makwapala Cotton Station near Zoniba, the first record of this species for that region, where it apparently causes considerable destruction, for Dr. Butler found as much as 30 to 40 per cent of the heads badly deformed and partly or completely sterile as a result of cumulative reinfection through continuous planting. Also in March, 1927, among the abundant clumps of wild grasses growing in Mr. Evans's tobacco estate at Bulaki, Nyasaland, Dr. Butler found a few tufts showing the shredding of the leaves into the tangles of fibers that are characteristic of infection by oogonial stages of Sclerospora. The host grass as determined at the Kew Herbarium proved to be *Eragrostis aspera* Nees., and material was brought back by Dr. Butler for further study.

On his return Dr. Butler and Mr. E. A. Mason, of the Bureau of Mycology, examined and compared these collections and found that the fungus on Eragrostis differed sharply from *Sclerospora graminicola* in that the resting spores were smaller and had bumpy protrusions from the oogonial wall. Knowing my interest in this genus, Dr. Butler generously gave me the material and hospitably extended to me the laboratory facilities of the Mycological Institute where part of this study was carried on in the summer of 1930. The fungus proves to be a strikingly distinct species that in the bumpy protruding wall and the small size of the resting spores and oospores is absolutely distinct from any species described hitherto. Also, it is known only on this host from this locality, and its parasitism of this species of grass adds another genus and species to the ever-growing list of gramineous

[1] Contribution No. 114, Laboratories of Cryptogamic Botany, Harvard University.

[2] Weston, W. H., Jr. A new Sclerospora from Fiji. Phytopath. 19: 961–967. 1929.

[3] *Ibid.* A new Sclerospora from Australia. Phytopath. 19: 1107–1115. 1929.

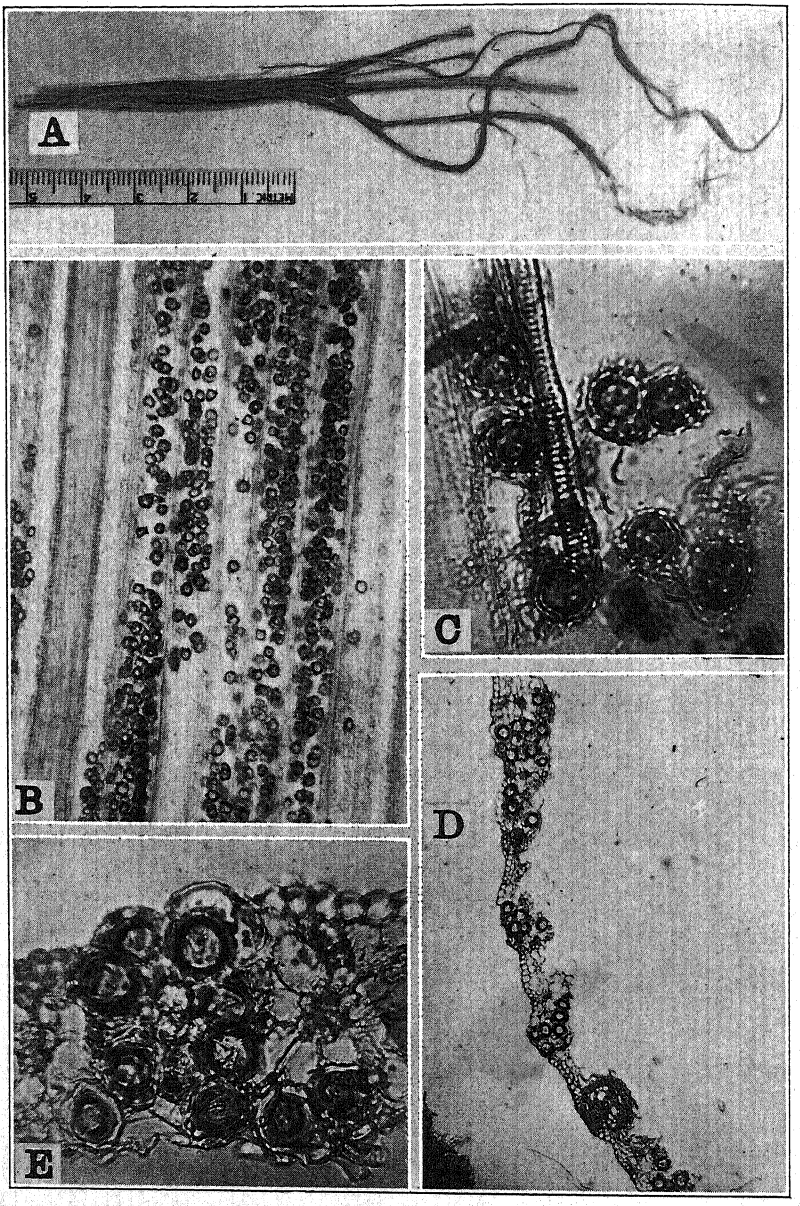


FIG. 1. A. Portions of leaves of *Eragrostis aspera* showing the disintegration of the leaf tissue into tangled fibers as a result of infection by *Sclerospora Butleri*. ×1. B. Surface view of a heavily infected portion of leaf tissue cleared in lacto-phenol to show the crowded masses of resting spores within. ×40. C. Bit of leaf tissue teased out to show the resting spores. ×175. D. Cross section of a slightly infected leaf showing the occurrence of the resting spores within. ×40. E. Similar cross section more highly magnified to show the position of the resting spores. ×175.

hosts for the Sclerosporas. In the hope that because of these features the fungus may prove of interest to others, a brief discussion of it is presented in the following note.

EFFECT ON THE HOST

On the Eragrostis the fungus produces a general withering and browning of the leaf until in later stages of development the mesophyll tissue between the bundles is destroyed (Fig. 1, B, D, E) and the leaf shreds out into tangled snarls of fiber. (Fig. 1, A.) This symptom is well known as characteristic of most of the species of Sclerospora in their oogonial phase. In this Nyasaland material the slender, elongate discolorations of the leaf tissue that appear early in the development of the parasite have almost disappeared and no traces of any conidial phase are present, nor did Dr. Butler encounter any in the field.

CHARACTERISTICS OF THE FUNGUS

Of the mycelium, as would be expected in an oogonial phase so far advanced in development, only fragmentary traces remain here and there.

The resting spores are developed in the mesophyll in great numbers, disorganizing the tissue until they come to occupy practically all of the space between the fibrovascular bundles. In leaves properly cleared (Fig. 1, B) they can be seen in this position before the disintegration of the epidermis of the leaf permits them to scatter. These resting spores, as is characteristic of the genus, comprise a heavy outer wall, which morphologically is the thickened modified wall of the oogonium, closely enclosing the single thick-walled oospore within. (Figs. 1, C, and 2, B.)

The oogonial wall is relatively thick, ranging from 4 to 10 μ (inclusive of protrusions), approximately $\frac{1}{5}$ of the total diameter of the resting spore. The surface is furnished with the blunt, rounded protrusions that are characteristic of the species and unknown for any other Sclerospora described hitherto. These protrusions, which may be rounded conical to tapering papillate or finger-like (Fig. 2), vary from 2 μ in width at the base and 2 μ high, up to 3 or 4 μ at the base and 4 or 5 μ high. They are solid, not hollow; pallid, almost hyaline, in marked contrast to the dark body of the oogonium wall; and distinctly modified in substance, reacting differently to treatment with stains or with chloro-iodide of zinc. The color of the main shell of the oogonial wall is variable in the many hundreds of spores examined but ranges from a pallid golden to a dark amber or resin, *i.e.*, from the Mars Yellow or Raw Sienna to the Sudan Brown or Antique Brown of Ridgway.[4]

[4] Ridgway, Robert. Color standards and color nomenclature. 43 pp.; 53 col. pls. Washington, D. C., 1912.

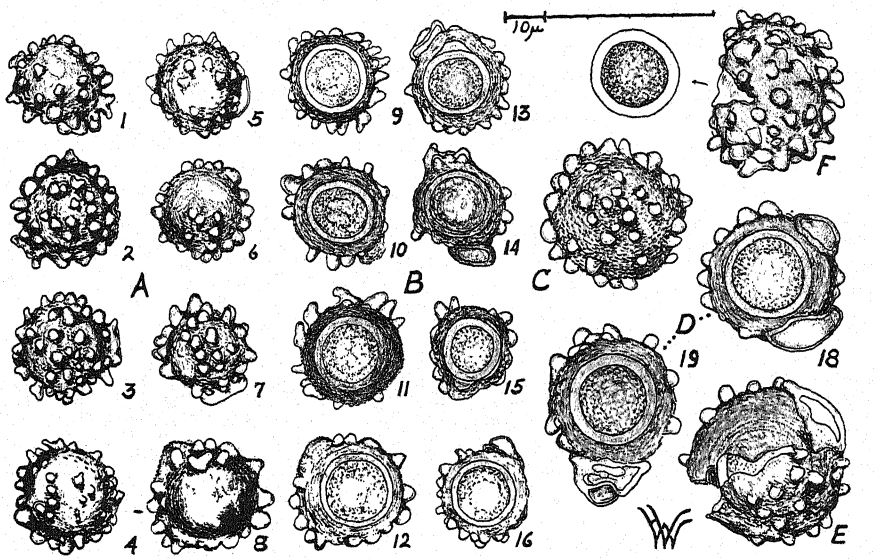


FIG. 2. A. Resting spores of *Sclerospora Butleri* in surface view, showing the pale, bullate, or papillate protrusions from the surface of the dark oogonial wall. ×500. B. Resting spores seen in optical section showing the hyaline protrusions, the dark body of the oogonial wall itself closely enveloping the spherical pallid oospore within. ×500. C. Surface view of a typical resting spore more highly magnified. ×700. D. Two resting spores seen in optical section showing, adhering to the exterior of the spore, structures that in 18 might be antheridia but, in 19, seem to be portions of the stomatal structure from the epidermis beneath which the spore was developed within the tissue of the leaf. ×700. E. Resting spore cracked open to show the relative size and position of the oospore within. ×700. F. Resting spore cracked open, freeing the enclosed oospore that now lies beside it. ×700. Camera-lucida drawings, scale in microns.

This outer oogonial wall fits closely to the single oospore within and enclasps it so adherently that only with difficult and careful manipulation can the oospore be freed. (Fig. 2, E and F). Rarely slight gaps are noticeable between the oospore wall and the inner surface of the enveloping oogonium coat that encloses it. (Fig. 2, B, 13, 15). The oospore is usually in an approximately centric position, as the oogonial wall is of almost even thickness throughout. (Fig. 2, B). Very seldom do any fragments of an oogonial stalk remain attached; a contrast to *Sclerospora noblei* in which this is a common occurrence. Very rarely also are adherent fragments seen that might be interpreted as traces of attached antheridia (Fig. 2, B, E); more commonly such fragments seem to be adherent remains of stomatal guard cells from the adjacent epidermis of the leaf. (Fig. 2, D).

In size the resting spores vary greatly, but, in general, the outer dimensions, including the protrusions, range from 29 to 40.9 μ, with average diameters of from 33 to 36.9 μ perhaps most commonly encountered (Table 1).

The single oospore within, almost exactly spherical, and the smallest of any Sclerospora yet known, ranges from 15 to 26.9 μ in diameter, most frequently 19 to 22.9 μ (cf. Table 1). The wall is smooth, hyaline, from 2 to 3 μ in thickness, most frequently averaging $2\frac{1}{4}$ to $2\frac{1}{2}$ μ. The content shows a finely granular matrix, hyaline or of grayish protoplasmic color, with occasional oil globules that do not, however, make any definite pattern of associated droplets nor any definitely eccentric or centric position of single drops. Attempts to induce germination of the resting spores have all been unsuccessful thus far.

TABLE 1.—*Measurements of the diameters of 100 resting spores of Sclerospora Butleri, n. sp., and of 200 oospores, arranged in size classes to show frequencies*

Diameters of resting spores		Diameters of oospores	
Classes μ	No. of resting spores in 100	Classes μ	No. of oospores in 200
21 to 22.9	1	15 to 16.9	2
23 to 24.9	1	17 to 18.9	18
25 to 26.9	0	19 to 20.9	73
27 to 28.9	1	21 to 22.9	92
29 to 30.9	10	23 to 24.9	14
31 to 32.9	13	25 to 26.9	1
33 to 34.9	25		
35 to 36.9	24		
37 to 38.9	15		
39 to 40.9	9		
41 to 42.9	1		

IDENTITY OF THE FUNGUS

Even though the material is scanty and consists of resting spores alone, as no conidial phase is known, the fungus stands out as quite distinct from any of the Sclerosporas hitherto described. The small size of the oospores and the bullate or papillate character of the exospore are sharply distinguishing features. The size of the oospores, ranging from 15 to 26.9 μ and most commonly 19 to 22.9 μ, is distinctively smaller than that of the oospores of *Sclerospora noblei,* hitherto the smallest known, with their diameter most frequently 25 to 26.9 μ. Coupled with the small size of these oospores of this new Sclerospora on Eragrostis in Africa is the relative thickness of the wall, which commonly is 2 to 3 μ, approximating that of oospores in species of much greater diameter and twice as heavy as those of *S. noblei,* which commonly are 1 to 1.5 μ in thickness.

It is the bluntly rounded to papillate protrusions from the surface of the exospore, however, that most sharply distinguish this species. By these

protrusions the surface of the exospore is given an appearance wholly unlike the irregularly ridged, angularly winged, polygonally faced to patchily scabrous contour typical of most species. Nor does the bullate or papillate surface of the spores of this new Sclerospora approximate that of the spores of *Sclerospora noblei,* whose dark, bluntly rounded, knobby projections are of the same dark color as the remainder of the oogonial wall, few in number and sufficiently large and irregular in many cases to render the whole spore unsymmetrical. In this new species from Africa, on the contrary, the protrusions are pallid, in marked contrast to the dark body of the oogonium wall, numerous, often close together, and, although varying in size, in general follow the contour of the oogonium, so that the profile outline inclusive of the projections is usually approximately spherical. Not only do these structural characters mark this species as distinct, but also the host on which it occurs, *Eragrostis aspera,* is new to the long list of grasses parasitized by various species of Sclerospora.

For these reasons, even though the writer is reluctant to describe as new a fungus with an incompletely known life history, it seems necessary to recognize this as a separate species. It is therefore described as follows:

Sclerospora Butleri, n. sp. Oogonium (outer portion of the resting spore) approximately spherical to irregularly subspherical; 29 to 40.9 μ in diameter, most frequently 33 to 36.9 μ; pallid golden to dark amber or resin color (Mars Yellow or Raw Sienna to Sudan Brown or Antique Brown, Ridgway[5]), the dark body of the oogonial wall of relatively even thickness ranging from 4 to 10 μ exclusive of protrusions, the surface set with numerous bluntly rounded, papillate to finger-like protrusions, pallid, almost hyaline, in contrast to the dark body of the oogonial wall, and approximately 2 μ in width at the base by 2 μ high, to 3 or 4 μ at the base and 4 or 5 μ high. Oospores occupying an approximately central position within the enveloping oogonium, the oospores regularly spherical, small, most frequently from 19 to 22.9 μ in diameter, the wall hyaline, usually 2 to 3 μ in thickness, content with a finely granular matrix, hyaline or grayish, with one or several oil droplets not arranged in any definite pattern.

Germination of the resting spores not yet observed. Conidial or sporangial phase unknown.

Occurring on *Eragrostis aspera* Nees. at Bulaki, Nyasaland, Africa, collected by Dr. E. J. Butler in March, 1927.

Specimens deposited in the Farlow Herbarium, Harvard University.

Oogonia sphaerica vel irregulariter subsphaerica, 29–40.9 μ diam., saepissime 33–36.9 μ, flava vel flavo-brunnea ("Mars Yellow" vel "Raw Sienna" deinde "Sudan Brown" vel "Antique Brown," Ridgway); tunica

[5] See footnote 4.

colorata circumcirca aequali, 4–10 μ crassa protrusionibus exclusis; protrusionibus hyalinis vel pallidis, papillosis vel rotundato-conicis, 2–5 μ altis, ad bases 2–4 μ diam.; oosporis centricaliter dispositis, globosis, saepissime 19–22.9 μ diam., episporis hyalinis 2–3 μ crassis intus granulosis hyalinis vel griseis, guttulis 1–∝ irregulariter dispositis. Germinatione oosporae nondum visa. Conidiis vel sporangiis ignotis.

Hab. in foliis *Eragrotis aspera* Nees. Bulaki, Nyasaland, Africa, coll. E. J. Butler, Mar. 1927.

DISCUSSION

Our knowledge of this Sclerospora, even though at present rather scanty, involves certain interesting features.

In the first place, the occurrence of this Sclerospora on *Eragrostis aspera* adds another representative to the list of gramineous hosts known to harbor species of this destructive genus. Of the distribution of the fungus on this host little can be said, for, while it is known at present only from this one locality in Bulaki, Nyasaland, the host on which it occurs, as indicated by the collections in the Kew Herbarium and by the ranges of geographical distribution given in various floras, is common through tropical Africa, occurring from the seacoast up to over 6,000 ft., from Natal and the Cape, up through the Belgian Congo, Tanganyika, Kenya, the British East Africa Protectorate, Zambesia, Sudan, Uganda, Belgian Congo, Nigeria, Rhodesia, Angola, Sierra Leone, Kamerun, the Gold Coast, up into Somaliland, Abyssinia, and Eritrea. It seems wholly probable, therefore, that more intensive mycologic collecting in these regions may discover this species of Sclerospora elsewhere throughout the range of its host. It is even possible it might be found as well on other related hosts in this region or elsewhere in Nyasaland, from which as yet only this fungus and the wide-spread *Sclerospora graminicola* collected by Dr. Butler on *Pennisetum typhoideum* have been reported.

In the second place, the fungus is of interest in itself because of the distinctive pallid, papillate protrusions from the surface of the exospore, a structural feature quite in contrast to the contour and surface sculpturings of any species of Sclerospora known hitherto. So unusual is this wall character for a member of this genus that it might be argued that this fungus may not prove to be a Sclerospora at all. The small size of the entire resting spore and of the oospore contained within, the papillate or bullate protrusions from the exospore surface, the lack of any recognizable extensive peronosporaceous mycelium, and the lack of any conidial or zoosporangial nonsexual reproduction typical of the downy mildews, might be advanced as evidence that this is one of the Chytridiales allied perhaps to Cladochytrium or Physoderma. Yet, the size, although smaller than that

of any other known Sclerospora, is approached by that of *Sclerospora noblei,* while the bullate surface of the exospore, although a character never encountered hitherto in any species of Sclerospora, is not in itself a valid reason against inclusion in this genus, since, in species of other genera of the Peronosporales, such as Albugo, strikingly different surface sculpturings may occur. The lack of any conidial phase in the present scanty material may mean no more than that such a phase has not yet been found, for, until recently, no such stage had been discovered for *S. noblei,* while in the case of *S. farlowii* and *S. northi* the justice of their inclusion in Sclerospora is unquestioned, even though no conidial phase has yet been discovered for either of them. Also, the absence of a recognizable peronosporaceous mycelium is not unusual in mature oogonial material of most species of Sclerospora when leaves are in an advanced stage of shredding, as in this. Moreover, the fact that this fungus parasitizes one of the Gramineae and produces ultimately the shredding of the leaves into tangled fibers is an indication of its being one of the Sclerosporas, a conclusion supported still further by the fact that the resting spore has the general Sclerospora structure of a heavy exospore closely enveloping the single oospore within. Obviously, there is great need for further study of the life history of this species, and it is to be hoped that some one strategically located in the region where it occurs will carry on investigations to increase our scanty knowledge of the fungus and to settle any doubts as to its identity and relationship.

As to the possible economic importance of this fungus, little can be said at the present state of our knowledge concerning it. It might, of course, have the beneficial effect of helping to keep down this grass in its rôle as a troublesome weed in coffee plantations and other cultivated areas. On the other hand, it should be noted that from the Gold Coast *Eragrostis aspera* is reported to be a forage grass of value, being relished by horses at all times, although eaten by cattle only when it is young, while in Rhodesia large quantities are grown and sold in the market for hay and the racing stables use it commonly. If this Sclerospora proves as seriously destructive to this grass as other Sclerosporas are to many other valuable cultivated or forage Gramineae, it may well become an important disease. Moreover, the possibility of its transferring to other more valuable grasses cannot be overlooked. The genus Eragrostis, occurring in the tribe Festuceae of Hitchcock under the subfamily of Poacoideae, comprises over a hundred species, both tropical and temperate, some of which are of importance, while among the 30 to 40 genera in the same tribe there are others, such as Bromus, Phragmites, and Arundo, already known to be hosts for species of Sclerospora.

SUMMARY

This paper discusses a Sclerospora causing browning, withering, and ultimate shredding of the leaves of *Eragrostis aspera* in Nyasaland, where it was collected by Dr. E. J. Butler.

In its resting-spore stage, the only reproductive phase yet known, the surface shows distinctive, rounded, bullate or papillate protrusions from the darker exospore, while the oospores within are the smallest of any Sclerospora yet encountered, even smaller than those of *Sclerospora noblei.* The species is described as *S. Butleri,* n. sp., and its distinguishing features of structure and surface configuration are discussed.

That the host, *E. aspera,* is an addition to the list of grasses parasitized by species of Sclerospora is noted, and questions of distribution and possible economic importance are discussed.

LABORATORIES OF CRYPTOGAMIC BOTANY,
HARVARD UNIVERSITY.

STEM CANKER OF CROTALARIA SPECTABILIS CAUSED BY DIAPORTHE CROTALARIAE, N. SP.

GEORGE F. WEBER

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INTRODUCTION

In the summer of 1930, at Gainesville, Florida, *Crotalaria spectabilis* Roth. plants were found bearing cankered areas on the stems which apparently had been caused by a fungus. Cultures of a fungus were obtained from the pith under the cankered areas and were observed for a year, but no signs of sporulation developed. During 1931, additional isolations were made and the fungus obtained was indistinguishable from that obtained during the previous season. Blossoming Crotalaria plants, grown in pots in the greenhouse, were inoculated with mycelium of the fungus and in 10 days stem lesions developed that, after 2 weeks, were typical of those on the plants in the field. Pycnidia developed on the inoculated plants, which contained spores typical of the genus Phomopsis. Careful observations in the field revealed similar fruiting structures on diseased plants and buried perithecia with long hair-like necks containing Diaporthe-like spores on old Crotalaria stems of the previous season's growth. The pathogenicity of the organism has been established, its imperfect and perfect stages have been connected in culture and through inoculation experiments, and the disease and the parasite are described herewith.

THE DISEASE

While studying Crotalaria diseases (3), in 1930, stem canker was discovered in a small planting of *C. spectabilis* at Gainesville. During the following season it reappeared in a more epidemic form, and further observations showed that it was present in all plantings of *C. spectabilis* in the vicinity of Gainesville. It was also found in the vicinity of Leesburg and Lake Alfred. The percentage of infected plants at Leesburg and Lake Alfred was about the same and relatively small, but at the station where Crotalaria had been planted on the same land during consecutive years and the old stems from previous seasons were used as a mulch for trees bordering on the field the percentage was very high. In this planting more than 90 per cent of the plants were infected and 30 per cent were killed by blossoming time. Other fields visited in 1931 showed from 15 to 20 per cent infected plants. The disease has been found only on *C. spectabilis,* at present the most extensively grown species in the State.

Description. The disease appears first in summer when the host plant is in bloom. At this time the plants are from 3 to 6 ft. high and the stems are from ½ to 1½ in. in diameter at the soil line.

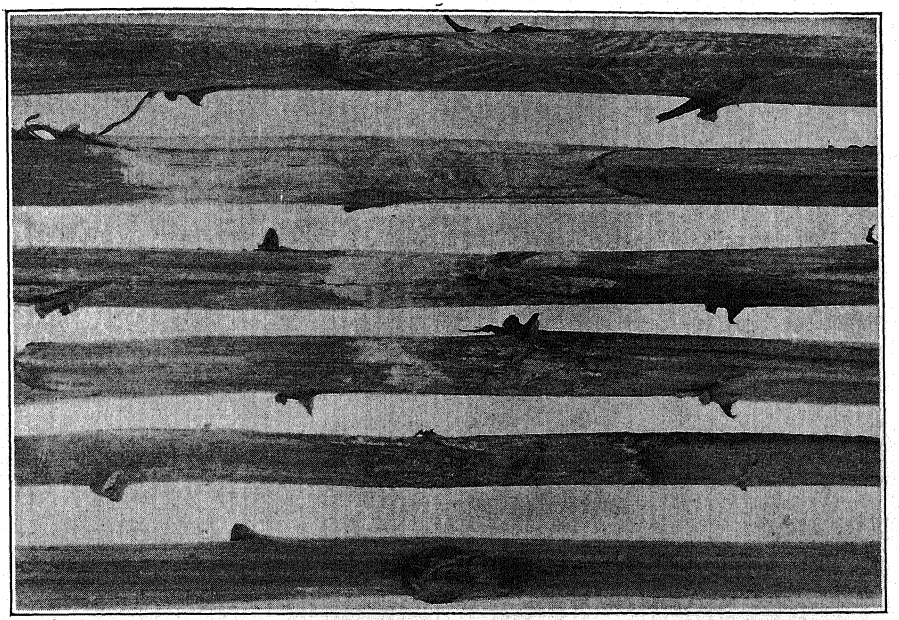


FIG. 1. Stem cankers on Crotalaria caused by *Diaporthe crotalariae*, showing various stages of development.

The stem lesions (Figs. 1 and 2, A) usually occur a foot or more above the soil line and below the branches of the inflorescence on plants in thick stands, while they occur at the soil line or within a foot of it in the more bushy plants of thin stands.

With few exceptions infection of the main stem occurs through leaf or branch scars. Having entered the stem, the fungus invades all parts of it rapidly, involving an area of 6 to 8 in. on each side of the infection point in a few days. As a rule, the stems are invaded for a distance of several inches before the parts of the plant above show symptoms of decline. The young lesions are a wood-brown (4) color, at first, but, as they enlarge, they become zonated by the formation of grayish white bands of mycelium underneath the epidermis (Fig. 1). This type of zoning is very characteristic of the disease and becomes more pronounced until the plants are killed. On plants growing in thin stands that develop branches close to the ground, infection usually takes place around the base of the branches. Lesions on such plants are less extensive and less characteristically zoned, although infected plants usually are killed.

With few exceptions pycnidia develop sparingly on the lesions. (Fig. 3, A). Occasionally, a lesion was found thickly dotted with imbedded pycnidia, which became erumpent during wet weather, and conidia exuded



FIG. 2. A. *Crotalaria spectabilis* plants, showing the disease as whitish areas on the lower portion of a number of the stems. B. One-year-old Crotalaria stems used as mulch. On these the perithecial stage was found.

from them in yellowish tan tendrils or masses. Conidia were seldom found, however, until after the plant was killed.

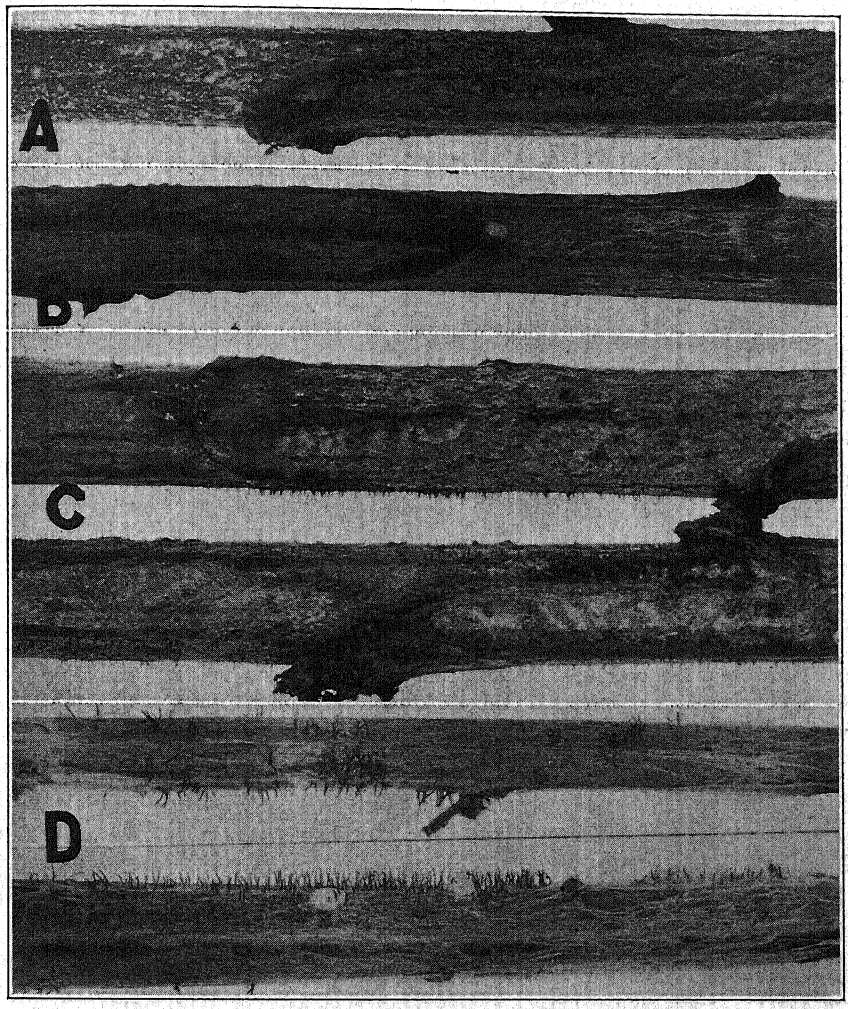


FIG. 3. Diseased Crotalaria stems. A. Pycnidia with spore horns exuding. B. Mycelium. C. Perithecia from nature. D. Perithecia developed in culture.

The perithecial stage of the fungus was found on current season's growth several months after it had been killed and on old stems of the previous season's crop. (Fig. 3, C.)

THE CAUSAL ORGANISM

Isolations. The first isolations were made from cankers on the stems. Considerable difficulty was experienced because of the loose porous pith,

in disinfecting the diseased material sufficiently to eliminate contaminants. Successful isolations were obtained, however, when the ends of the diseased stalks were recut in the laboratory and immediately sealed with melted paraffin, previous to disinfection in corrosive sublimate 1:1,000 for 15 minutes. The organism secured grew well on potato-dextrose agar, producing a dirty-white, flocculent, aerial growth characteristic of species of Phomopsis. A large number of isolations were made from these diseased areas in which the same fungus was obtained consistently. Cultures of this organism have been kept on potato-dextrose agar and transferred in the laboratory for a year and a half and no signs of sporulation have developed. On sterilized Crotalaria stems it produced pycnidia and pycnospores sparingly.

In the summer of 1931 spore horns were observed exuding from pycnidia on lesions on plants in the field. These specimens were examined in the laboratory and were found to contain both the small ovoid spores and stylospores characteristic of the genus Phomopsis. Cultures obtained from the infected pith underneath stem lesions and from single pycnospores obtained from the exuded spore horns were indistinguishable and resembled in every respect the fungus isolated the previous year from the diseased areas on the plant.

After the disease was definitely found to be caused by a species of Phomopsis, a search was made on old diseased plants for a Diaporthe that might possibly be the perfect stage of the fungus. Several piles of one-year-old Crotalaria stems, used as mulch around fruit trees, showed that a good percentage of the stems bore perithecia of a Diaporthe. (Fig. 2, B.) Single ascospore isolations were made and the resulting mycelial growth on potato-dextrose agar was comparable to that produced by the fungus isolated from infected host tissue and from pycnospores. The cultures obtained from ascospores produced perithecia on potato-dextrose agar after 3 weeks, which were comparable with those on old Crotalaria stems. The mature asci and ascospores thus produced in culture were indistinguishable from those developed in nature on Crotalaria stems. Sterilized Crotalaria stems were inoculated with potato-dextrose-agar cultures obtained from mycelium in the host tissue, from pycnospores, and from ascospores. After several weeks the stems inoculated with mycelium originating from ascospores showed perithecia typical of Diaporthe and similar to those found in nature on old Crotalaria stems. (Fig. 3, D.) Careful examination of these cultures a week later showed that all of them were producing both pycnidia and perithecia containing mature pycnospores and ascospores, respectively. The stems inoculated with cultures obtained from isolated mycelium and pycnospores produced pycnidia but not perithecia.

Pathogenicity. Blossoming Crotaliaria plants, growing in greenhouse pots, were inoculated with mycelium, pycnospores, and ascospores of the fungus. The mycelium growth on the surface of agar plates was cut in strips for convenience in inoculating plants. The spores were used in water suspensions. The inoculum in all instances was placed on the leaf scars where leaves were freshly removed or in incisions made through axillary buds. The inoculated areas were then closely covered with adhesive tape, which extended about an inch above and below the wound. The disease began to show on the stems beyond the tape-covered areas after 14 days. The data obtained from the experiments are shown in table 1.

TABLE 1.—*Results of inoculations on Crotalaria spectabilis with Diaporthe crotalariae, December 1931*

Source of inoculum	Number of plants	
	Inoculated	Diseased
Mycelium	18	18
Pycnospores	12	10
Ascospores (nature)	14	9
Ascospores (culture)	15	10
Sterile water	6	0

These inoculation experiments show that the mycelium and spore forms of the fungus are pathogenic on *Crotalaria spectabilis.* Characteristics of the disease produced by each of the inoculations were similar, and, after 3 or 4 weeks, they could not be distinguished, except by the labels. The plants inoculated with mycelium developed a higher percentage of infection and the resulting disease developed more quickly than that present in plants inoculated with ascospores or pycnospores. Four weeks after inoculation pycnidia appeared on several of the plants inoculated with pycnospores and ascospores. The diseased portions of these stems were removed and placed in moist chambers in the laboratory and after several weeks perithecia developed on all of them. The perithecia were imbedded between the wood and bark and had long hair-like beaks comparable to those found in nature and those developed on agar and sterilized Crotalaria stems. Both spore forms that developed on the inoculated plants were comparable with those found in nature.

Thus, briefly summarizing, the pycnidia and pycnospores found in nature were comparable with those produced in culture from spore isolations. The ascigerous stage developing on the host from pycnospore inoculations was the same as that found in nature. Likewise, the perithecia and ascospores developing from isolated ascospores on the host and in culture were

the same as those found in nature. The ascigerous stage was produced from the imperfect stage through the host and the imperfect stage from the ascigerous stage. The ascigerous stage was not developed in artificial culture from the isolated mycelium or pycnospores.

Taxonomy. A careful study of the literature failed to reveal a similar fungus on any species of Crotalaria. However, it was learned that Diaporthe has been reported on plants in closely related genera, but these are apparently quite different from the species discussed herein. Other diseases of economic importance caused by similar fungi have been reported by Harter and Fields (1), Jenkins (2), and Wolf (5) on sweet potato, rose, and citrus, respectively. Since *D. citri* (Faw.) Wolf is very common in most citrus groves and *C. spectabilis* is grown extensively as a cover crop in these plantings, some connection might be possible, but cross inoculations with the citrus organism failed to produce infection on the crotalaria.

It has been concluded after careful study that the disease on Crotalaria is undescribed and is caused by a hitherto undescribed fungus. Consequently, the parasitic fungus is described as follows:

Diaporthe crotalariae, sp. nov.

Synonym: Pycnidia—**Phomopsis crotalariae,** n. f. nom.

Pycnidial stage: Pycnidia on killed areas of stems imbedded under epidermis, irregular to subglobose, scattered, black, thick-wall, simple or chambered, pseudoparenchymatous, rupturing irregularly, erumpent at maturity, 200–450 μ in diameter. Pycnospores: ovate to elongate, hyaline, guttulate, unicellular, 5.9–10 × 1.9–2.8 μ, average 7.73 × 2.88 μ. Sporophores: simple hyaline tapering to apex, 15.4–26.6 μ long. Stylospores: not common, slender, hyaline, hooked, 16.6–30.94 × 1.8–2.3 μ.

Perithecial stage: On dead stems of the host 3 months to 1 year after being killed, single or gregarious, imbedded in stromata between bark and wood, globose to lens-shape, black, pseudoparenchymatous, walls 220–380 μ in diameter. Beaks protruding through bark, 1–2 mm. long, mostly 1–1¼ mm. tapering, black with brownish rounded tip, hairy on surface, ostiole about ¼ diameter of beak, apical pore circular. Asci: subsessile, elongate-clavate, sides often irregular, apex slightly thickened with pore, average 34.01 × 6.85 μ, 44 per cent measure 34.2 × 6.84 μ, aparaphysate. Ascospores: irregularly biseriate, hyaline, ellipsoid, obtuse, bicellular, slightly constricted at septum, each cell biguttulate at maturity, 9.12–12.31 × 2.28–3 μ, average 10.95 × 2.5 μ. (Fig. 4.)

Produces stem and branch cankers on *Crotalaria spectabilis.* Pycnospores found on recently killed parts, while ascigerous stage found on same parts after 3 to 6 months. Type material in the herbarium of the Florida Agricultural Experiment Station, Gainesville, Florida.

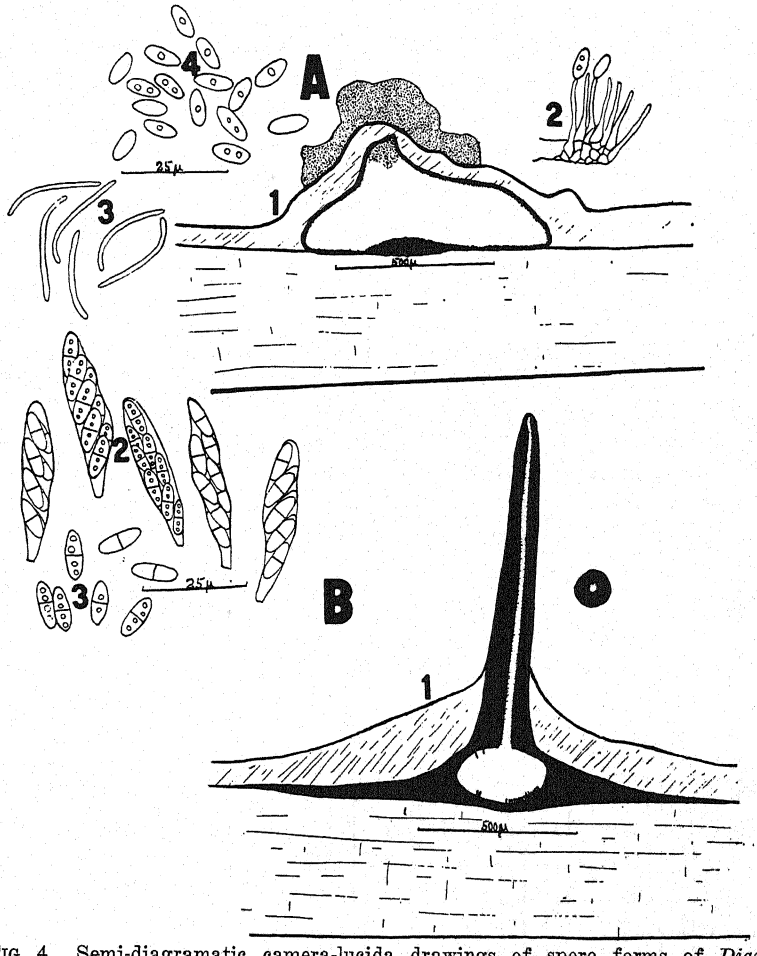


FIG. 4. Semi-diagramatic camera-lucida drawings of spore forms of *Diaporthe crotalariae*. A. (1) Pycnidium and exuded pycnospores; (2) sporophores; (3) stylospores; (4) pycnospores. B. (1) Longitudinal section of perithecium and cross-section of beak; (2) asci; (3) ascospores.

SUMMARY

A stem disease of *Crotalaria spectabilis*, distributed in central Florida, is described for the first time.

In certain fields at blooming time 30 per cent of the plants have been killed and 90 per cent infected by the disease.

The disease has been reproduced in inoculation experiments by using isolated mycelium, pycnospores, and ascospores of the fungus as inoculum.

The ascigerous stage has been obtained from the imperfect stage through inoculated plants. The imperfect and ascigerous stages have been obtained both in culture and through inoculated plants from the ascigerous stage.

The imperfect stage of the fungus is described as *Phomopsis crotalariae*, n. f. nom., and the ascigerous stage is described as *Diaporthe crotalariae*, sp. nov.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

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THE PATHOLOGY OF TOBACCO BLACK SHANK

FREDERICK TAYLOR WOLF[1]

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INTRODUCTION

Nearly every study that has been made of the tobacco disease, black shank, caused by *Phytophthora nicotianae* Breda de Haan, has made reference to the rapidity with which infected plants wilt and die. In most cases only 4 or 5 days are required to produce a wilting that involves the entire plant, and, after 2 or 3 more days, the plants succumb. There appears to be too little destruction of the cortex and pith to result in rapid collapse and subsequent death of the plant, especially since the xylem tissues are intact or only slightly invaded. Nolla (15), in his account of the histology of the disease as it occurs in Puerto Rico, states that the hyphae, which are both intra- and intercellular, invade parenchyma tissues and cause their dissolution.

Up to the present, the pathological studies of this disease, both as it occurs in the East Indies and in this country, do not account for the rapidity with which infected plants wilt. It seemed advisable, therefore, to inquire into the possibilities of the production of toxins, enzymes, acids, or alkalis to explain this phenomenon. The literature on these subjects is too voluminous to be summarized herein, but the following investigations are deemed pertinent to the present study.

Although the matter of toxin production by plant pathogens is not yet completely understood, it has received considerable attention. As long ago as 1913, Hutchinson (13) performed experiments with *Bacterium solanacearum* E. F. Sm., the organism causing tobacco wilt, which led him to believe that the production of toxic substances is, in the last analysis, responsible for the symptoms exhibited by diseased plants. Coons (5), in 1916, concluded that the wilting of potatoes caused by *Fusarium oxysporum* Schl. is attributable to poisons generated by this fungus. Additional evidence adduced by Haskell (9), in 1919, discredited the previously accepted theory advanced by Smith (19) 20 years before, which held that plants infected with species of Fusarium wilt because the mycelia in the xylem block the tracheae, thus shutting off the supply of water to the leaves. Of this, Haskell says, "The writer has examined the stems of many plants microscopically and found no instance of a stoppage of the trachea sufficient to shut off the passage of sap. . . . The theory that one or more toxines are responsible is much more plausible." Young and Bennett (23), work-

[1] The writer wishes to express his appreciation to Dr. F. A. Wolf, at whose suggestion the present problem was undertaken and with whose help and guidance it was completed.

ing with the same disease, definitely established the fact that *F. oxysporum* produces a substance toxic to potato plants. Gross (8) found that the killing of potato plants by *F. eumartii* C. W. Carpenter is not due to a blocking of the tracheae.

In 1924, Farrell (7) reported that the wilting of tomatoes in Kansas by *Fusarium lycopersici* Sacc. is caused by an enzyme. White (22) and Haymaker (10), however, working with the same disease, concluded that this wilting is the result of the production of toxic substances. Bewley (3) reported that the so-called "sleepy disease," another tomato wilt, is due to an exoenzyme secreted by *Verticillium albo-atrum* Reinke and Berth. Picado (17) subsequently showed that the symptoms of sleepy disease are caused by a chronic poisoning of the plant tissues.

Ajrekar and Bal (1) suspected that toxins are produced by the cotton-wilt organism, *Fusarium vasinfectum* Atk. In 1926 Rosen (18) demonstrated that 2 separate and distinct toxins, one an alkaline substance and the other a salt in the form of a nitrite, are produced by the cotton-wilt pathogen. Brandes (4) stated that toxins are probably a proximate cause of banana wilt, *F. cubense* E. F. Sm. The green mold, *Penicillium expansum* Link, was described by Barnum (2) as being capable of secreting substances toxic to plants. Fahmy (6) found that *F. solani* Schl. excretes a toxic material that "produces a rapid wilting of cut bean stems when these are placed in the filtrate of the fungus." The halos surrounding lesions produced by tobacco wildfire, *Bacterium tabacum* Wolf and Foster, have been attributed by Johnson and Murwin (14) to a toxin.

In his experiments with *Fusarium solani,* Fahmy (6) noted the increase in the degree of alkalinity accompanying a corresponding increase in the age of the culture, but he does not regard this alkalinity as being responsible for wilting. In the case of stem rots caused by *Sclerotium rolfsii* Sacc., Higgins (11) states that the death of cells subjacent to those invaded by the fungus is due to the toxic action of oxalic acid secreted by the fungous hyphae. His paper contains a summary of investigations concerning the production of oxalic acid by various bacteria and fungi. Smith (20) concluded that the pathogenicity of *Botrytis cinerea* Pers. to lettuce plants is due to the formation of oxalic acid, while Peltier (16) expressed the opinion that the toxic substance is an organic acid other than oxalic.

The results of an inquiry into the production of toxins, enzymes, and acids by *Phytophthora nicotianae* are presented as an explanation of the rapid wilting of tobacco by this pathogen.

TOXIN PRODUCTION IN CULTURE

Potato broth was found to be a favorable substrate for a rapid mycelial growth by *Phytophthora nicotianae.* A very satisfactory growth also was

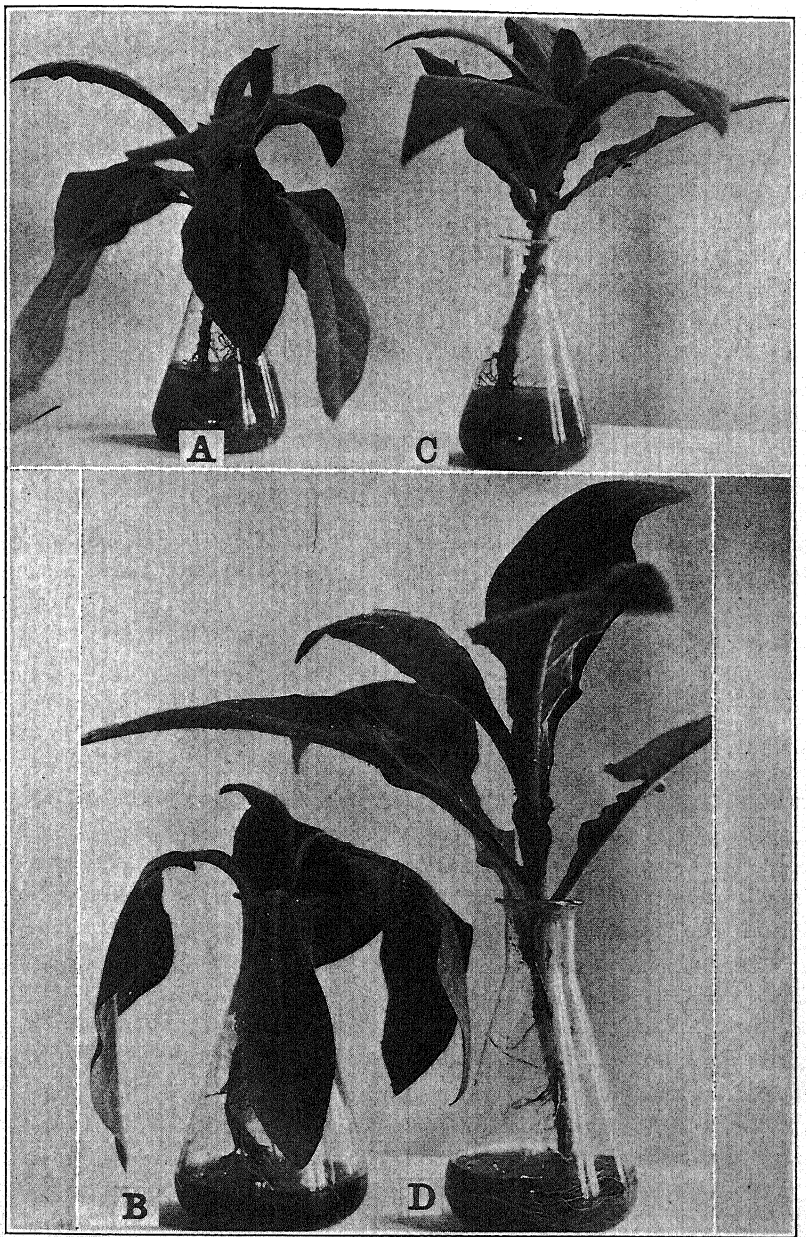


FIG. 1. A and B. Wilting of tobacco plants. A. In filtrate from 13-day-old potato-broth culture of *Phytophthora nicotianae*. B. In filtrate of material from infected tobacco plants. C. andD. Controls. C. In noninoculated potato broth. D. In filtrate from material from healthy tobacco plant. A and C photographed after 1 hour and B and D after 1½ hours.

obtained with flasks of Richards' solution diluted by ½ with distilled water. Toxin production was first tested after the organism had grown for 9 days in flasks containing potato broth. The broth was filtered through a fine grade of filter paper on a Büchner funnel. Microscopic examination showed the absence of the organism from the filtrate. A healthy tobacco plant was uprooted, washed free of soil, and its roots were immersed in this filtrate. A similar plant, placed in noninoculated potato broth adjusted to the same pH concentration, served as a check. In 45 minutes the plant in the filtrate was wilted, while the control remained normal. When this experiment was repeated with potato broth in which the pathogen had grown for 13 days, a period of 30 minutes sufficed to cause marked wilting, while the control remained turgid. Figure 1, A and C, shows these 2 plants an hour after the beginning of the experiment.

The same procedure was used to test the presence of toxins in cultures grown in Richards' solution. In 17-day-old cultures, 30 minutes sufficed to produce wilting. Plants placed in noninoculated Richards' solution as controls remained turgid. Because of the rapidity with which plants wilted when placed in filtrates from inoculated potato broth and Richards' solution, it seems probable that wilting was accomplished by toxic materials produced by the organism.

TOXIN IN DISEASED PLANTS

To obtain additional evidence of the production of toxin by *Phytophthora nicotianae*, infected tobacco plants that had been grown in the greenhouse were used. The diseased portion of the stems was removed and cut into pieces to facilitate maceration with a mortar and pestle. Sand that had received washings with water and sulphuric acid was used in the mortar as the abrasive agent. After grinding, a small quantity of distilled water was added to the macerated material, which then was stored at 34° F. for 18 hours. Extraction for only a few minutes sufficed to cause marked wilting.

The material was then filtered through a Büchner funnel, and a healthy tobacco plant was placed in the filtrate. A similarly prepared filtrate from healthy tobacco stems served as a control. After 45 minutes, the plant in the filtrate from diseased stems was badly wilted, while the control remained normal. These plants are shown in figure 1, B and D, as they appeared after 1½ hours.

This experiment was repeated with infected field-grown plants. In this case 1 hour was required to wilt the plant whose roots were immersed in the filtrate from black-shank lesions, while the control remained turgid.

Hursh (12) has pointed out that too much emphasis is being placed on the wilting of plants following their immersion in filtrates of various fungi,

since in many cases the wilted plants will regain their normal appearance on being removed from the filtrate and placed in fresh water. With this in mind, wilted plants were removed from the filtrates of cultures of *Phytophthora nicotianae* and placed in fresh water, but, when badly wilted, they did not recover. Recovery seems to be conditioned by the length of time plants are exposed to the toxin and by the degree of collapse.

NATURE OF THE TOXIN

Macerated tissues from diseased plants were allowed to stand in distilled water for 18 hours at a temperature of 34° F. Crystals of $MgSO_4$, which may be used to precipitate proteins, were added to the liquid obtained by filtration of this material. As the concentration of $MgSO_4$ approximated saturation, perceptible floccules were formed. When this material was again filtered, the floccules were retained on the filter paper. After several washings with water to remove any $MgSO_4$, this filter paper, along with the precipitate it contained, was placed in a flask of distilled water.

A healthy tobacco plant with roots immersed in this solution was severely wilted when examined 24 hours later. The toxic substance, therefore, was precipitated with the proteins.

Seventeen-day-old cultures of *Phytophthora nicotianae,* grown on Richards' solution, were used to test the effect of temperature on the stability of the toxin. One portion was stored for 48 hours at 34° F. Another portion was boiled for 5 minutes. After the solutions had regained room temperature, they were filtered through a Büchner funnel, and a healthy tobacco plant was placed in each. One 19-day-old culture and a flask of noninoculated Richards' solution, in which plants were placed, served as controls. The plant with roots immersed in the boiled filtrate wilted in 15 minutes, while 25 minutes were required to wilt the plant in the filtrate that had been subjected to a temperature of 34° F. The plant in the 19-day-old culture wilted badly in 15 minutes, and the one placed in the noninoculated Richards' solution remained normal. This evidence would seem to indicate that the toxic substance is not affected by temperatures within the range 34° to 212° F.

In testing for volatility of the toxin, a 20-day-old flask culture on Richards' solution was distilled for 30 minutes, and the gases that came off were collected in distilled water. They were colorless and of a pungent, disagreeable odor. After cooling, a plant was placed in the distillate and another in the liquid remaining in the distilling flask. The latter wilted severely in 8 minutes, while the plant in the distillate remained turgid, showing that the toxin is nonvolatile.

ACID PRODUCTION BY THE ORGANISM

A quantity of potato broth, which had an initial reaction of pH 6.5, was used as the nutrient solution in this experiment. Portions of this medium were adjusted with HCl and NaOH to concentrations of 4.0, 4.8, 7.2, 8.2, and 8.8, and 1 portion was left unadjusted. After tubing and autoclaving, the solutions were inoculated with *Phytophthora nicotianae*. Colorimetric tests of changes in reaction were made at intervals, as shown in the following table:

TABLE 1.—*Changes in acidity of cultures of Phytophthora nicotianae*

Initial reaction	Reaction after			
	4 days	6 days	8 days	9 days
4.0	4.0	3.6	3.4	3.4
4.8	4.6	4.6	4.4	4.2
6.5	5.6	5.0	4.6	4.6
7.2	6.2	5.6	5.0	4.6
8.2	6.8	5.6	5.0	4.6
8.8	7.8	7.2	5.4	5.0

The cultures the initial concentration of which were pH 4.8 and 6.5 made the most rapid growth. This accords with the findings of Tisdale and Kelley (21), who state that pH concentrations of 4.4 to 5.1 are most favorable for growth.

In view of the fact that many fungi are known to produce oxalic acid, it seemed desirable to test for the presence of oxalates. When a mixture of the filtrate and concentrated sulphuric acid was heated and the gases given off were passed through baryta water, there was no evidence of the formation of barium carbonate. Also, when the filtrate was neutralized with NaOH and a solution of $CaCl_2$ was added, no precipitate of calcium oxalate was formed. Both tests show, therefore, that the acids formed in these cultures are not oxalic.

INFLUENCE OF PH CONCENTRATION ON WILTING

In view of the fact that plants wilted when placed in filtrates of the fungus, it seemed desirable to determine if the pH concentration of the filtrate was a cause of this wilting. The fungus was allowed to grow in half-strength Richards' solution for 17 days. The mycelial mat was removed, and the solution filtered through a Büchner funnel. After filtration, portions of the nutrient solution were adjusted to pH concentrations of 4.4 and 8.6. Healthy tobacco plants were placed in flasks containing these adjusted filtrates. Examination after 30 hours showed both plants severely

wilted, while those placed in flasks of noninoculated Richards' solution, at the same concentrations, showed no wilting in a corresponding period. This is interpreted to show that neither organic acids produced by the pathogen nor the pH concentration of the media employed is responsible for the wilting of tobacco.

UTILIZATION OF CARBOHYDRATES

In unpublished data Carlos[2] has shown that the black-shank organism digests the middle lamella and the secondary cellulose membranes. The secretion of enzymes other than those responsible for these phenomena was tested by the use of carbohydrate media. Standard starch-agar plates were poured, inoculated with *Phytophthora nicotianae,* and allowed to stand for 3 days. When the plates were flooded with iodine, a colorless area appeared surrounding each colony, demonstrating the digestion of starch by the enzyme diastase (amylase) secreted by the fungus.

To study the possibility of the digestion of sugars by *Phytophthora nicotianae,* broths consisting of 2 per cent peptone and 2 per cent of dextrose, maltose, sucrose, or lactose, were made up and inoculated. One portion consisting of 2 per cent peptone alone was inoculated to serve as a check. The digestion of these sugars was determined colorimetrically by the change in hydrogen-ion concentration.

In dextrose and maltose, a change in reaction of 1.0 was produced during a 5-day period, and in sucrose a decrease of 0.8, indicating that these sugars are readily digested by the fungus. The digestion of dextrose, maltose, and sucrose necessitates that the fungus produce zymase, maltase, and invertase. In lactose and in peptone broth, on the other hand, the change within the given period amounted to only 0.2.

SUMMARY

The rapid wilting and collapse of tobacco plants infected with *Phytophthora nicotianae* are due to a toxin. This toxin may be extracted from black-shank lesions and is also present in cultures in which the organism has been grown. This toxin, which is thermostable and nonvolatile, is probably protein in nature.

Acids other than oxalic are produced by the black-shank fungus during the decomposition of tissues and in culture media. These acids do not account for the wilting, however.

Phytophthora nicotianae secretes enzymes that enable it to utilize the middle lamella, the secondary membranes, starch, sucrose, dextrose, and maltose.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA.

[2] Carlos, T. P. The black-shank disease of tobacco. Unpublished thesis, Duke University Library.

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FIELD OBSERVATIONS OF NEEDLE RUSTS OF SPRUCE IN MINNESOTA

RALPH M. LINDGREN

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In the summer of 1927 a needle rust of spruce appeared in epidemic form in northwestern Minnesota. The regional distribution of the disease in the State corresponded to the range of the swamp evergreen heath plants, Labrador tea, *Ledum groenlandicum* Oeder; leatherleaf, *Chamaedaphne calyculata* (L.) Moench; and bog rosemary, *Andromeda glaucophylla* Link. These plants became heavily infected with the alternate stages of the causal organisms later in the summer. The infected spruce was the cause of considerable concern to foresters, nurserymen, and owners of ornamental spruce plantings because epidemics of a similar nature had been infrequent, and little information was available on the nature and extent of the resulting damage. The prevalence of the disease during 1927 on ornamental as well as on native spruce, growing under natural conditions, afforded an excellent opportunity to add to the meager information concerning such epidemic attacks.

NOTES ON INFECTION

Immature aecia were first reported on spruce on July 14. Shedding of the spores began towards the latter part of July and continued for 3 weeks in August. Although infection was limited to the current year's growth, aecial production, especially in the case of native black and Colorado blue spruce, growing in areas of heavy attack, was so abundant that the foliage in mass was yellow. The aecial pustules occurred in irregular rows, sometimes definitely 2, on the under side of the needle, and varied in number from 3 to 9 but were usually 5 to 6. Trees of all ages were attacked, infection being heaviest in the leaders and upper portions of small trees under 8 ft. in height. In the larger trees, infected needles were confined for the most part to the lower portions of the crown. The relative susceptibility of several species of spruce growing side by side in forest nurseries was observed to be as follows: susceptible—black spruce, *Picea mariana* (Miller) Britton, Sterns, and Poggenberg, and Colorado blue spruce, *P. pungens* Engelmann; moderately susceptible—white spruce, *P. glauca* (Moench) Voss; resistant—Black Hills spruce, *P. glauca albertiana* (S. Brown) Rehder, and Norway spruce, *P. excelsa* Link. No attempt was made to substantiate these field observations on susceptibility by conducting controlled inoculation experiments on spruce.

The heath plants, leatherleaf, Labrador tea, and bog rosemary were usually found in the immediate vicinity of infected spruce, although in the

case of one nursery that contained rusted trees, the nearest plants found were ½ mile distant. Examinations were made of these plants at intervals during the month of August. The small red to yellow uredinial pustules were first noticed on August 16 on leatherleaf growing in the vicinity of heavily rusted black spruce. By the latter part of August this stage had become prevalent on both leatherleaf and Labrador tea, being hypophyllous in the case of the former and predominantly hypophyllous but occasionally epiphyllous on Labrador tea. Comparatively little infection was found on bog rosemary, even where heavily rusted plants of the other 2 species were in close proximity. Uredinial production continued through the months of August and September and was heaviest on those plants growing close to or under diseased spruce. Infection was most common on the thin small leaves that had not yet become leathery and were relatively free of tomentum. An examination of infected areas made in March, 1928, revealed only uredinia on old leaves. Clinton[1] reported having found uredinia in January on living old plants and believed them to be capable of overwintering.

NATURE AND EXTENT OF INJURY TO SPRUCE

Infected needles were being shed even before the aecia had ceased to liberate spores. Where heavy infection occurred, as on small trees and in the lower portions of the crown of large trees of black and Colorado blue spruce, it was estimated that from ¼ to ¾ of the current year's needles were lost by the end of the summer. Defoliation was slight in the case of white spruce and was of little or no consequence on Black Hills and Norway spruce. Whether such defoliation as occurred on black and Colorado blue spruce retarded growth or resulted in permanent injury was not established definitely. Reports received in 1928 from foresters and nurserymen indicated that the infected trees had recovered to the extent that little evidence remained of the heavy attack of the previous year. No killing or apparent deformation of the infected trees had occurred, although it was reported by several that growth had been retarded slightly. The effect of several successive years of defoliation could not be determined because only light attacks of the rust occurred in 1928 and 1929. It is probable that successive epidemics would result in pronounced retardation of growth, and possibly in permanent injury to the heavily infected plants.

The only known previous reports of injury to spruce caused by the needle-rust fungi are those of Clinton, who states that needles infected with *Melampsoropsis cassandrae* appeared as though they would be shed prematurely; and of Fraser, who describes killing of young plants by heavy pycnial infection in inoculation experiments with one of the rust fungi.

[1] Clinton, G. P. Heteroecious rusts of Connecticut having a Peridermium for their aecial stage. Conn. Agr. Expt. Sta. Ann. Rpt. 1907–1908: 369–396. 1908.

CAUSAL ORGANISMS

Three closely related and very similar needle-rust fungi have been described as commonly occurring on spruce, namely; *Melampsoropsis abietina* (Alb. and Schw.) Arthur, *M. ledicola* (Peck) Arthur, and *M. cassandrae* (Peck and Clinton) Arthur. These have as their alternate hosts evergreen swamp plants belonging to the heath family; principally leatherleaf, Labrador tea, and bog rosemary. The host relationships of 2 of these, *M. abietina* and *M. ledicola,* were established by Fraser[2] in 1910 and 1911 through studies conducted under both field and controlled conditions. The relation between *Peridermium consimile* and *M. cassandrae* was shown by Clinton[3] in 1908, and this work was later substantiated by Fraser. The aecial stages of all 3 of these organisms appear as needle rusts on spruce and very similar morphologically and, therefore, difficult to differentiate. The uredinial stages are more readily distinguished, those of *M. abietina* and *M. ledicola* being hypophyllous and epiphyllous forms, respectively, on Labrador tea; while the uredinial stage of *M. cassandrae* occurs as a hypophyllous form on leatherleaf.

In an effort to establish the identity and relative prevalence of the organisms concerned in the epidemic of 1927, examinations were made of the aecial and uredinial stages, both in the field and in the laboratory. The distinguishable differences in the aecial stages were found to be insufficient to permit a determination of the causal organisms on spruce. Since the uredinial stages of all 3 organisms were found on the heath plants, there was evidence that all were concerned to some extent. The heaviest infection occurred on leatherleaf, which suggested that *Melampsoropsis cassandrae* was at least common and probably most prevalent. On Labrador tea, the uredinial sori were predominantly hypophyllous, indicating a greater prevalence of *M. abietina* than of *M. ledicola.*

A number of inoculations of leatherleaf and Labrador tea were made in the field and in the greenhouse with the purpose of obtaining supplementary evidence on the relative prevalence of the several rust fungi. A search for a rust-free area was unsuccessful; consequently, plants growing in areas of light infection had to be used. In the field inoculations, 10 apparently noninfected plants of each species growing *in situ* were sprayed with fresh aecial material collected from black and Colorado blue spruce. Within 20 days all 20 plants showed infection, the uredinial sori being hypophyllous in all cases and more abundant on leatherleaf than on Labrador tea. Noninoculated plants in the immediate vicinity of the sprayed ones were either free of infection or showed light attacks only on the lower sides of the leaves.

[2] Fraser, W. P. Cultures of some heteroecious rusts. Mycologia 3: 67–74. 1911.
[3] *Loc. cit.* See footnote 1.

For the greenhouse studies, 40 plants of each species, together with the moss and substratum in which they were growing, were taken to the University Farm and potted immediately. Thirty plants of each species were sprayed with aecial material collected from infected spruce in the field, and 10 were kept as controls. A number of the plants soon showed evidences of loss of vigor as a result of transplanting, and several had withered before the incubation period of 21 days was completed. Of the 60 inoculated plants, 7 leatherleaf and 2 Labrador tea developed uredinial pustules on the lower sides of the leaves. Of the controls, all except 1 leatherleaf remained free of rust, infection in this 1 case probably having occurred while the plant grew in the field.

The meager infection results obtained in the inoculation studies permit no definite conclusions. It is interesting to note, however, that they tend to strengthen field observations, which indicated that *Melampsoropsis cassandrae* was very common on spruce and that *M. ledicola* was of little significance.

SUMMARY

A needle rust of spruce appeared in epidemic form in Minnesota during the summer of 1927. The relative susceptibility of spruce, growing in forest nurseries, was observed to be as follows: susceptible, black and Colorado blue spruce; moderately susceptible, white spruce; resistant, Norway and Black Hills spruce. Defoliation in the case of heavily infected plants amounted to from $\frac{1}{4}$ to $\frac{3}{4}$ of the current year's needles. Reports received in 1928 indicated that no killing or permanent deformation of the infected trees had resulted.

Field and laboratory examinations of the uredinial stages of the causal organisms on heath plants indicated *Melampsoropsis cassandrae* to be very common; *M. abietina* to be fairly common; and *M. ledicola* to be of little significance. Inoculation studies on leatherleaf and Labrador tea tended to strengthen the above conclusions as to the relative prevalence of the several fungi.

CONSISTENT MUTATION OF HELMINTHOSPORIUM SATIVUM ON A NO-NITROGEN MEDIUM

GLENN E. PAXTON

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Numerous workers have studied environmental factors involved in mutation of fungi. Stevens (5), Christensen (1), Leonian (3), Mitra (4), and others have shown that the type and amount of medium greatly influences the frequency of sector formation. Christensen (2) has shown certain temperatures to be more favorable than others.

The effect of nutrition on mutation has been studied in preliminary experiments, and evidence is presented here that mutations may be produced consistently in *Helminthosporium sativum* P., K., & B. on a standard medium.

The culture of *Helminthosporium sativum* studied by the writer was isolated from a barley leaf showing typical spot-blotch lesions that had been collected at Davis, California, in 1931. The plates were incubated at room temperature (18–25° C.) and examined after 2 to 3 weeks.

Czapek's medium was used in order to obtain the normal growth of the fungus. It was made up as follows: 0.5 gm. $MgSO_4$, 0.5 gm. KCl, 1 gm. KH_2PO_4, 0.01 gm. $FeSO_4$, 2 gm. $NaNO_3$, 30 gm. sucrose, and 20 gm. of agar in 1,000 cc. distilled water.

It was found that mutations or sectors occurred consistently when the sodium nitrate was omitted from Czapek's medium. This will be designated as the minus-N medium. Different depths of medium were used in Petri dishes with no difference in results. The usual amount employed was 20 cc. Single-spore or mass transfers on this medium produced similar colonies.

The results of 5 series of tests may be summarized as follows: On minus-N agar all of the 47 colonies from mass transfers and all the 91 single-spore colonies produced sectors, whereas among 44 colonies from mass transfers on Czapek's agar 3 produced sectors; among 103 colonies from single spores only 9 produced sectors.

Small white tufts of sterile mycelium were often produced on the colonies, but only the fan-shape sectors were taken into consideration in the results. The number of sectors per plate on the minus-N medium varied from 2 to 12, with an average of 5. The sectors grew faster than the remainder of the colony and were usually whiter and sporulated more sparingly, as shown in figure 1, A.

Sectors occurred occasionally on Czapek's agar, but all except one were unlike those that occurred on the minus-N medium, in that most of them were essentially sterile white mycelium. This difference was especially noticeable when the mutants were grown for comparison on Czapek's agar.

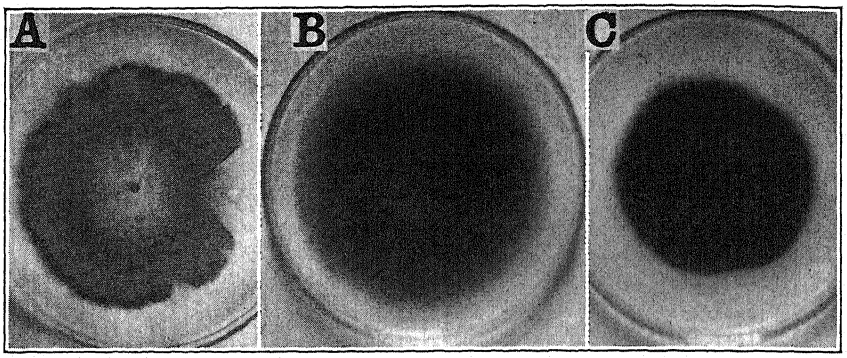


FIG. 1. A. Three-week culture of *Helminthosporium sativum* on minus-N medium showing sectors. B. Fifteen-day culture of the mutant growing on Czapek's agar. C. Fifteen-day culture of the original fungus growing on Czapek's agar.

The sectors that occurred on the minus-N medium were of many types, one of which invariably occurred on every plate. This type of mutant was studied in detail and is shown (Fig. 1, B) growing on Czapek's agar, in comparison with the original fungus on the same medium (Fig. 1, C). As compared with the normal, the mycelium of the mutant was darker, grew faster, the sporulation was heavier, and much more variation was evident in the length and shape of the spores. The spores of the mutant were darker brown, shorter, and more rounded at the ends. Five hundred spores averaged 55×24 μ, while the spores of the normal fungus averaged 72×22 μ.

Mass and single-spore transfers were made from the minus-N to Czapek's agar from the sectors and from the normal region between the sectors of the colonies. Change had occurred only in the transfers from the sectors.

When the spores were taken from the normal region of the colony and again grown on the minus-N medium, sectors occurred in every trial. Colonies taken from the sectors and grown on the minus-N medium have sectored only twice in about 100 trials. No sectoring of the mutant has occurred during 5 generations on Czapek's agar. Colonies from some of the other types of sectors not considered in this paper have, however, sectored still further on the minus-N medium and on Czapek's agar. Some have shown a partial reversion to the normal fungus.

The effect of the 2 media on anastomosis of hyphae was tested by growing the normal fungus on slides covered with a thin layer of agar. There was abundant hyphal fusion on both the Czapek's and the minus-N agars and no noticeable difference was observed. The same was true in observations made on many colonies growing in Petri dishes.

It has been found that the addition of ½ gm. of $NaNO_3$ per liter to the minus-N medium was sufficient to prevent most of the sector formation. When both sucrose and nitrogen were omitted from the Czapek's medium no

sectors were formed. Among 20 cultures grown on minus-N agar with only 10 gm. of sucrose per liter, and 20 cultures grown on minus-N agar with 100 gm. of sucrose per liter, sector formation was not interfered with in any plate. In 10 cultures of the normal fungus, KNO_3 was substituted for $NaNO_3$ in Czapek's agar and no difference in results was obtained. The omission of sodium was apparently not a factor in sector formation.

Twenty colonies, each, of 2 other forms of *Helminthosporium sativum* were grown on the minus-N medium and every one produced sectors.

Other fungi, such as Penicillium, Alternaria, and a Macrosporium were grown on 20 plates, each of the minus-N agar, but no sectors were produced.

Mutation in *Helminthosporium sativum* appeared to be related to nutrition. Lack of nitrogen seemed to be an important factor in causing consistent mutation. If nitrates were present, not only were decidedly fewer sectors formed, but these were different in morphology. A slower growth rate, increased time factor, and accumulation of by-products in the absence of nitrogen may explain partly the phenomenon of sector formation. Sucrose also plays an important part because, when this was omitted, no sectoring occurred. Depth of medium did not affect either the number or type of sectors. Since anastomosis of hyphae was abundant on both media, it is improbable that it has any effect in causing sector formation.

SUMMARY

On Czapek's agar, *Helminthosporium sativum* produced only occasional sectors, while all colonies on Czapek's agar, containing no nitrogen, produced sectors, one type of which occurred on every culture and was distinctly different from the original colony. Subplants from this mutant have shown no tendency to revert to the original, even in the fifth generation. On Czapek's agar, lacking sucrose as well as nitrogen, no sectors were produced.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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Three New Hosts for Tylenchus dipsaci, the Bulb or Stem Nema.—Bulbs of *Colchicum speciosum album* (Hort.), imported from Haarlem, Holland, and intercepted at the Inspection House of the Bureau of Plant Quarantine in Washington, D. C., August, 1932, were found to be infested with *T. dipsaci* (Kühn, 1858) Bastian, 1865. As far as we are aware, this is the first time that this parasite has been reported from a Colchicum. About 20 per cent of the bulbs of the shipment were affected. Judging from our observations, the disease must be very detrimental to the Colchicum bulbs. (Fig. 1, A.) The symptoms somewhat resemble those produced by the same did not revive, but were otherwise excellently preserved.—G. STEINER and parasite in bulbous irises. In every instance observed the infestation began at the base of the bulb, destroying the basal or root plate. The first signs of the disease are yellowish or brown streaks, which gradually spread out on the surface but also penetrate the bulb more and more. To properly see these symptoms, the brown cover scale must be loosened as shown in figure 1, A. A more advanced stage of the disease presents areas of a grayish color penetrating the bulb quite deeply. It consists of destroyed tissues that soon completely decay and then form a black powdery mass. The progress of the disease is shown in stages reading from left to right, (Fig. 1, A).

In the fall of 1932 shipments of *Chionodoxa luciliae* Boiss. bulbs, intercepted in New York City, Philadelphia, and Detroit, and all originating from Holland, were found infested with *Tylenchus dipsaci.* This seems to be the first record of this nemic pest in Chionodoxa. The disease symptoms occur externally, as well as internally, since there are produced both surface scales and internal brown rings analogous to the brown-ring symptoms of narcissus. (Fig. 1, B.) The affected surface scales are shriveled and exhibit a brown to black discoloration.

In January, 1933, two herbarium leaves of foxglove, *Digitalis purpurea* L., from the Elm City Nursery, New Haven, Connecticut, collected in June, 1916, were received from G. P. Clinton of the Connecticut Agricultural Experiment Station. The leaves (Fig. 1, C) showed spots in which large numbers of *Tylenchus dipsaci* were found. *D. purpurea* was not known to be a host of this nemic pest. The symptons are somewhat unusual for this nema, being more of the type produced in certain hosts by *Aphelenchoides fragariae.* Probably the parasites invaded the leaves under wet conditions from the under surface, entering through the stomata all over the leaves and forming thereby numerous infection centers simultaneously. The *T. dipsaci* specimens, having been in dry herbarium preservation for 16½ years, EDNA M. BUHRER, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

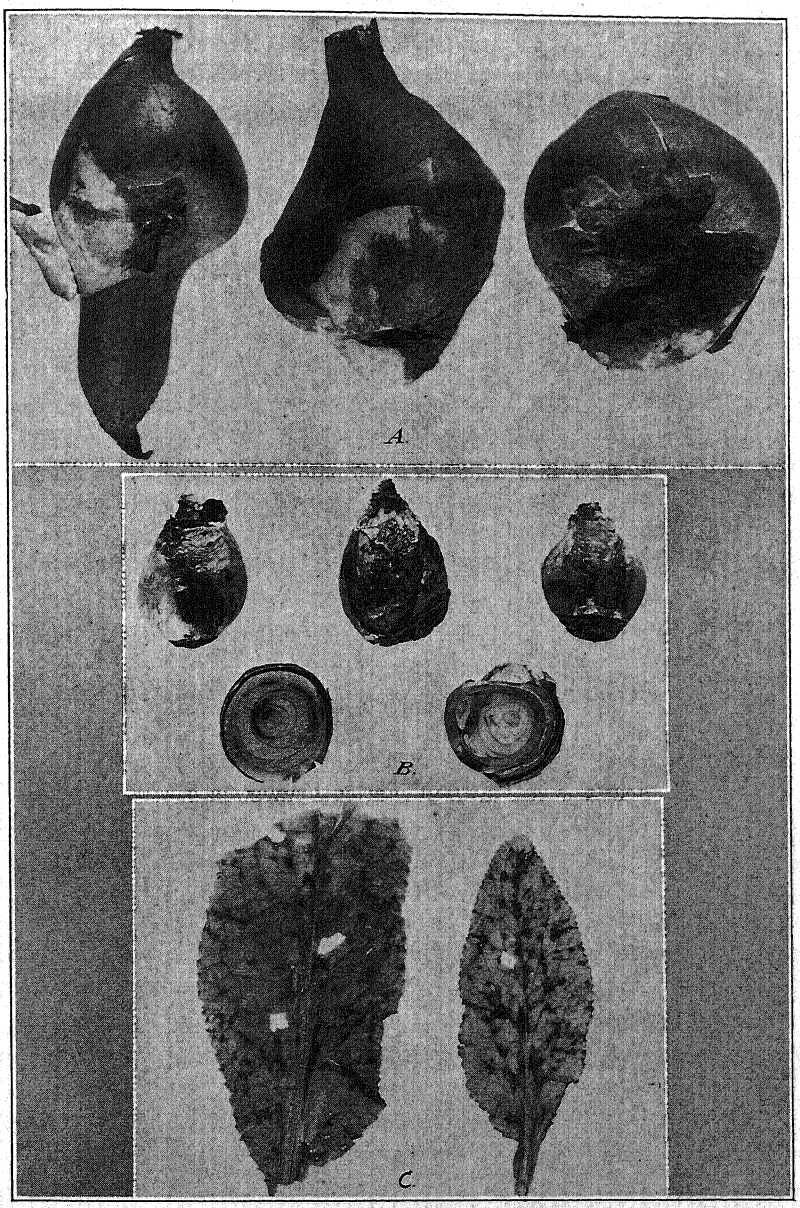


FIG. 1. A. *Colchicum speciosum album* bulbs infested with *Tylenchus dipsaci*. Progress of the disease shown in stages reading from left to right. B. *Chiondoxa luciliae* bulbs infested with *Tylenchus dipsaci* showing external and internal areas of infestation. C. *Digitalis purpurea* leaves infested with *Tylenchus dipsaci*.

Unusual Disease Symptoms Produced by Aphelenchoides fragariae.—Recently, some *Chrysanthemum hortorum* (Hort.) plants, which proved to be infested by *Aphelenchoides fragariae* (Ritzema Bos 1891) Steiner and Buhrer 1932,[1] were received from a nursery in Ohio. The disease symptoms were not those commonly observed on chrysanthemums when attacked by this nematode, *i.e.*, brown or yellow spotting of the leaves. Instead, the plants were a full and healthy green but exhibited defective leaf spread, some leaves being almost filiform, reduced, and abnormally crinkled, the leaf stem often swollen (Fig. 1, *a*). In some instances stems and leaves were dwarfed, crinkled, and bunched (Fig. 1, *a, d*), producing, with a shortened swollen stem, what might be called the "cauliflower" type of disease, not previously observed in chrysanthemums (Fig. 1, *b*). Stems may even be blind (Fig. 1, *c*). The affected plants were apparently of luxuriant, fast growth, the tissue being sappy and rather soft, a fact that may account for the unusual symptoms. *A. fragariae* is known to produce cauliflower symptoms in strawberry plants, the explanation offered in this case being similar to that just mentioned. Various host plants of the bulb or stem nema (*Tylenchus dipsaci*), if in luxuriant, sappy growth, also react to such a nemic infestation by production of swollen curved stems and crinkled leaves (cauliflower type), whereas under dry conditions and slower growth no such symptoms are developed.—G. STEINER and EDNA M. BUHRER, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

A Potato Disease.—Potatoes, both in greenhouse and field at Ithaca, New York, became affected in late August, 1932, with a serious disease, which had the general appearance of top necrosis (acronecrosis) and was so considered for some weeks. The disease was observed first in species hybrids of domestic by *Solanum demissum, antipoviczii,* etc., from Mexico but soon spread to a number of domestic varieties, Rural New Yorker, Green Mountain, Katahdin, Triumph, and others, as well as to several tuberous and nontuberous species of Solanum from Mexico, also to *Physalis* spp., to *Nicandra physaloides,* to *Datura stramonium,* to pepper (*Capsicum* sp.), and to tomato (Lycopersicum).

Symptoms appear first in the apical leaflets. The leaflets begin to cup downward and show numerous darkish areas on the underside. The tips of shoots begin to turn brown and from day to day become browner and very brittle. In the meantime the undersides of the leaflets assume a glassy appearance followed by a bronzing of the whole area. The leaf petiole becomes brittle and the leaf breaks off with a touch. Axillary shoots pass through these stages rapidly and die completely. The stalks and fully

[1] Synonyms of this form are: *Aphelenchus subtenuis* Cobb 1926, *A. olesistus* Ritzema Bos 1893, and *A. ritzema-bosi* Schwartz 1911.



FIG. 1. *Chrysanthemum hortorum* infested with *Aphelenchoides fragariae*. Outstanding symptoms marked with arrows, as explained in text.

matured leaves may remain green for a long time or they may die rather promptly, apparently depending on the age of the plant when first attacked. First-year potato seedlings usually are killed outright. The long stolons of Mexican species and of many of the hybrids are very brittle on affected plants as compared with healthy ones. Tuber formation is checked as soon as the disease is contracted.

The disease is readily communicated by grafting and by leaf mutilation with diseased material so that in very many respects it has the characters of a virus disease. Mention of the disease in a session of The American Phytopathological Society elicited the information that a mite probably is responsible for the trouble, and this proves to be the case. The creatures are microscopic in size and scarcely seem abundant enough on the foliage to account for the profound changes induced in the plants. The mite involved is of the genus Tarsonemus, but the specific identity is a matter for further study that is being done by Wm. Blauvelt, of Cornell University. Mr. Blauvelt's familiarity with the Cyclamen mite permits him to say definitely that it is a different species.

With the exception of minor variations, the disease corresponds with the brief description of Carpenter[1] concerning an outbreak of mites in Hawaii and with that of Mann *et al.*[2] of an outbreak in India. The latter authors found the disease a very serious one in the "summer" crop of potatoes in the whole Poona district of India, and Kulkarni[3] later found peppers over wide areas in India severely injured by the same organism.

The mites yield to fumigation with naphthalene.[4] It is a relatively simple matter to rid a greenhouse of the pests, but their occurrence in the field is a more serious matter. Lime sulphur solution and sulphur dust have been used effectively in fields of India. No outbreak of this or a similar pest in the field seems to have been recorded for continental United States of America. It may be that the Ithaca outbreak of 1932 is sporadic, but it would be unwise to minimize the possible danger. Every effort will be made to avoid further trouble in 1933.—DONALD REDDICK, Cornell University, Ithaca, New York.

[1] Carpenter, C. W. A new disease of the Irish potato. Phytopath. 8: 286–287. 1918.

[2] Mann, Harold H., S. D. Nagpurkar, and G. S. Kulkarni. The "tambera" disease of potato. Agr. Jour. India 15: 282–288. 1920.

[3] Kulkarni, G. S. The "murda" disease of chilli (Capsicum). Agr. Jour. India 17: 51–54. 1922.

[4] Hartzell, Albert, and Frank Wilcoxon. Naphthalene fumigation at controlled concentrations. Jour. Econ. Ent. 23: 608–618. 1930.

BOOK REVIEW

Root Nodule Bacteria and Leguminous Plants. By E. B. Fred, I. L. Baldwin, and Elizabeth McCoy. 343 pp. University of Wisconsin Studies in Science No. 5, Madison, Wisconsin. 1932.

While this book is primarily a review of the entire literature pertaining to root nodules and root-nodule bacteria of leguminous plants, it is thoroughly interspersed with authoritative opinions and deductions. The authors have brought to light from available history material that it is rarely the pleasure of most of us to see. Reference to the use of legumes as crops and food is carried back about 6,000 years, and evidence is presented to show that the seeds of the lentil, a legume, were used by the builders of the pyramids. The writings of Cato in the 2nd century B. C. give one of the first indications of an appreciation for the leguminous plant as a soil benefactor. The necessarily fragmentary ancient history of legumes is followed by a discussion of their use in New World agriculture from the time of discovery to the present period and also includes some of the recently acquired archaeological data from Mayan and Pre-Incan explorations. This first chapter chronicles the classical experiments with legume bacteria by scientists of the 19th century, culminating in the work of Hellriegel, to whom credit for substantial progress in explaining nitrogen fixation by legumes is given.

Legumes are considered botanically in the 2nd chapter, and here, as well as in the 12th chapter, they are discussed from an economic standpoint. Covering a period from 1587, a summary is given of the conceptions of various observers of the nature and functions of root nodules of legumes. Space is also devoted to nonnodule-bearing legumes, to nodule-bearing nonlegumes, and to the normal leaf growths that harbor presumably essential bacteria. A continuance of the work on nodules is found in chapter 9 where their life history is carried through the infection thread stage, nuclear alteration, and the digestion of the bacteria in the cells.

Starting with chapter 4, methods of isolating and differentiating legume bacteria are presented, followed by a discussion of the known life history of the organism and its responses to environmental influences such as temperature, light, aeration, and composition of the substratum.

Of interest to pathologists is the part of chapter 10 concerning variations in physiological efficiency among nodule organisms that amounts to parasitism at times but is usually of a beneficial nature. The work on nitrogen fixation by a combination of the plant and its organism and by the organism alone brings the comment "that perhaps the effort to divorce the rôles

of the plant and bacteria in the process of nitrogen fixation is fundamentally wrong."

The literature on the influences that affect nodule production leads the authors to believe that there is much yet to be learned regarding environment in relation to nodule production. The last chapter is concerned with the practical application of nodule bacteria to soils and seeds; the various methods of transferring field soil are outlined; and an interesting history of the development of the artificial or pure-culture business in Europe and the United States is given with considerable detailed reference to patents issued in this connection. An appendix includes specific directions for the use of artificially prepared legume-bacteria cultures, a list of sources from which inoculating material of this type may be obtained, data on the extent of the distribution of such cultures, and the names of governmental agencies concerned in the regulation of the trade together with the laws through which they operate.

In addition to a wealth of information most of which it is not possible to mention here, the book contains many illustrations. It will be a great asset to scientific workers and, in view of its practical bent, should serve as an informational handbook to those interested in soil bacteriology from a commercial standpoint.

A bibliography of 1,058 references follows the appendix.—LEWIS T. LEONARD, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

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TWO LEAF SPOTS OF THE PEANUT (ARACHIS HYPOGAEA L.)[1]

NAOMI C. WOODROOF

(Accepted for publication August 26, 1932)

The peanut is an extensively grown and valuable crop in many parts of the world. Large acreages are grown in the southeastern United States, South Africa, parts of South America, the West Indies, Philippine Islands, Japan, India, Australia, Java, Italy, and in China. Seed from these widely separated regions have been exchanged, and as a consequence it is probable that diseases have become widely disseminated throughout most of these areas. The prevalence of peanut leaf spots is shown by the fact that they are mentioned in literature from all of these countries.

In 1928, during the course of some preliminary seed treatment tests, it was noted that there were 2 distinct leaf spots occurring in abundance on the leaves of peanut plants growing in the field at the Georgia Experiment Station. Further studies were begun to determine which of these spots was originally described by Berkeley (4) as *Cladosporium personatum* and if this was the same as that studied by Ellis and Everhart (10) when they changed the name of the causal organism to *Cercospora personata*. Fungi other than *C. personata* have been described as causing spotting of the leaves of the peanut plant, but most of these have been considered synonymous by subsequent writers. The present investigation is an attempt to clarify the resultant confusion by comparing the various descriptions and herbarium specimens and by comparing these with the 2 spots found in Georgia.

LITERATURE REVIEW

In the following literature review only those references are listed that give sufficient detail to enable the reader to determine rather accurately the systematic classification of the disease described. The writers who include in their papers a synonymy of the respective fungi are listed in table 1.

In 1875 Berkeley (4, p. 106) published the first description of a peanut leaf spot and gave the name *Cladosporium personatum* to the causal fungus. This was the result of a study of material collected by Ravenel in South Carolina in 1855.

[1] Published with the approval of Director as Paper No. 36—Journal series Georgia Experiment Station.

Ten years later Ellis and Everhart (10), after examining material collected in Alabama and South Carolina, transferred the fungus to the genus Cercospora. This change has been generally accepted and the name *Cercospora personata* used exclusively in preference to *Cladosporium personatum.*

In 1891 Atkinson (1, p. 43) examined material collected in Alabama and South Carolina that was reported to agree with specimen N. A. F. No. 2,480 collected by Professor Tracy and determined as *Cercospora personata* by Ellis and Everhart. Atkinson, however, reports a greater conidial length and more septations than are recorded by Ellis and Everhart. It is possible that Atkinson examined leaves infected by 2 different fungi, as he found the conidiophores to be amphigenous, whereas Ellis and Everhart describe the fungus as being hypophyllous.

In Raciborski's (18) description of *Septogloeum arachidis* appear 2 statements that become significant when compared with the illustrations and descriptions of later writers. These are, first, "*Das Mycelium des Pilzes breitet sich am stärksten zwischen den Schwammparenchymzellen aus und sendet in diese charakteristische gelappte Haustorien,*" and, second, "*Flecken konzentrisch gruppirte.*"

Hennings (12) described *Cercospora arachidis* from a study of material collected in Brazil and says he classifies it as a new species, because "*Von C. personata* (B. & C.) Ell. *durch die aufgeblasenen Flecke, die septirten Hyphen, die kürzeren, 3–6 septirten conidien der Beschreibung nach verschieden.*" It seems possible Ellis and Everhart failed to mention the fact that their fungus produced raised spots or that they covered this point in a statement regarding the densely tufted conidiophores. They also probably failed to observe that the conidiophores were septate in some instances. The variation in the conidial length does not seem great enough to be important. Later writers have commonly listed *C. arachidis* as synonymous with *C. personata.*

Zimmermann (29, Taf. 2, Fig. 9) illustrated the conidiophores and the conidia of *Septogloeum arachidis.* From these drawings and the accompanying description it was concluded that Zimmermann studied the same leaf spot as described by Raciborski. A comparison of Raciborski's collection with N. A. F. No. 2,480 shows that *S. arachidis* is the same as *Cercospora personata.*

Bancroft (3, pp. 52 and 55) reports both *Cercospora personata* and *Septogloeum arachidis* from the West Indies. The more complete description of *S. arachidis* may indicate that he was more familiar with descriptions of this leaf spot. His description of *C. personata* is similar to that of Ellis and Everhart. Possibly he knew of a second leaf spot that he believed described by these writers as *C. personata.*

TABLE 1.—*The writers who give or suggest synonymous relationships of the peanut leaf-spotting fungi are listed chronologically as follows:*

Authority	Date	Reference no. in cited literature	SYNONYMS (The first name given is that accepted by each writer)
Ellis and Everhart	1885	10	*Cercospora personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C.
Chevalier	1913	7, p. 72–73	*Septogloeum arachidis* Rac. *Cercospora personata* (B. & C.) E. & E. *C. arachidis* P. Henn.
Wolf	1914	27	*Cercospora personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C. *Cercospora arachidis* P. Henn.
Yates	1917	28, p. 315	*Cercospora personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C. possibly *Septogloeum arachidis* Rac.
Van Der Bijl	1920	5	*Septogloeum arachidis* Rac. *Cercospora personata* (B. & C.) E. & E.
Wakefield	1921	25, p. 299	*Cercospora personata* (B. & C.) Ell. *Septogloeum arachidis* Rac.
Sawada	1922	22	*Cercospora personata* (B. & C.) E. & E. *C. arachidis* P. Henn. *Septogloeum arachidis* Rac.
Maublanc	1924	17	*Cercospora personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C. *Septogloeum arachidis* Rac. *Cercospora arachidis* P. Henn.
Mason	1928	16	*Cercospora personata* (B. & C.) E. & E. *Septogloeum arachidis* Rac.
Curzi	1931	9	*Cercospora personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C. *Septogloeum arachidis* Rac. *Cercospora arachidis* P. Henn.
Solheim and Stevens	1931	24, p. 368–369	*Cercospora personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C. *Cercospora arachidis* P. Henn.
Saccardo	1886	21	*C. personata* (B. & C.) E. & E. *Cladosporium personatum* B. & C.

Heald and Wolf (11, p. 49) may have studied a mixed infection, but it is quite possible that they collected only specimens infected with the Cercospora producing long slender obclavate conidia that they illustrate as *C. personata.* They state that the conidiophores are amphigenous though more appear on the lower surface.

Butler's (6) description and illustrations of *Septogloeum arachidis* are similar to those of Zimmermann. They were both undoubtedly dealing with the same type leaf spot. Butler is the first writer to illustrate haustoria in the cells of the leaf tissue. This writer states that *Cercospora personata* also occurs in one province in India but did little damage.

Wolf (27) undoubtedly has described a mixed infection as *Cercospora personata*. His illustrations show slender obclavate conidia and 2 types of conidiophore tufts, 1 small and having a less definitely stromatic base, while those of the second are large and stromatic. The former was produced epiphyllously and the latter hypophyllously. The statement is made that the large definitely stromatic conidiophore tufts are not typical of the genus Cercospora. This writer considered *Septogloeum arachidis* as described by Butler from India to be a separate leaf disease of the peanut, though he was aware that Chevalier (7) had considered it identical with *C. personata*. Wolf considered *C. arachidis* synonymous with *C. personata*.

Shaw (23) and Mackenna (14, p. 85) reported in 1917–18 2 leaf spots of the peanut in India, 1 *Cercospora personata* and the other an unknown species of Cercospora. Neither writer described these 2 fungi.

Welles (26), in 1917, illustrated *Cercospora personata* from the Philippine Islands as having long, slender, obclavate conidia. A year later Reinking (19, pp. 176–177) described and illustrated the fungus of a leaf spot of peanuts that had short, thick, abruptly obclavate or cylindrical conidia and that was considered to be *Septogloeum arachidis*. In 1919 this same writer (20, pp. 114–115) referred to *C. personata* but stated that, "Further study will probably show that the Philippine leaf spot is also a species of Septogloeum." It is probable that he referred to the same fungus in both cases.

In 1917 Hori[2] (13, p. 26) described from Japan a Cercospora leaf spot of the peanut to which he gave the name *C. arachidicola*. The original description by Hori has not been read, but a comparison of a specimen collected by Hori, with the descriptions and illustrations by Sawada (22),[3] has enabled the writer to determine that *C. arachidicola* is the same as the long, slender-spore Cercospora commonly found on peanuts in southeastern United States that other writers have often confused with *C. personata*.

[2] Since this paper was submitted for publication a letter was received from M. Ishikawa inclosing a translation of Hori's description of *Cercospora arachidicola*, and also one of Sawada's paper. The translation of the former is particularly valuable because the writer has been unable to obtain a copy of the original in America. Hori's description is very brief, and his principal claim for priority lies in the type specimen which he prepared.

[3] The writer is very grateful to Mr. Geo. T. Akamatsu for an English translation of this reference.

Sawada's (22) illustrations of *Cercospora personata* are similar to those of Zimmermann, Butler, and Reinking, for *Septogloeum arachidis,* and of Maublanc and Bal for *C. personata.* His illustrations of *C. arachidicola* are similar to those of Welles, Heald and Wolf, and Wolf.

Maffei (15), in 1921, described a Cercospora with long, slender spores on peanut leaves collected in Italy, which he classified as a variety of *Cercospora arachidis* (*C. arachidis* var. *macrospora*). This writer considered *C. arachidis* as distinct from *C. personata.* No reference is made to Hori's description of *C. arachidicola.*

Only Hori and Maffei have actually described this species of Cercospora on the peanut; and, as Hori's description has priority, *C. arachidicola* must be accepted as the name of this fungus rather than Maffei's *C. arachidis* var. *macrospora.*

Bal (2), 1921, was the first writer to illustrate the fungus having thick, abruptly obclavate conidia under the name *Cercospora personata.*

In 1924 Maublanc (17) published drawings of *Cercospora personata* that are very like those of Zimmermann, Butler, Bal, Reinking, and Sawada, for *C. personata* and *Septogloeum arachidis.* Maublanc also expresses the opinion that this fungus possibly should not be classified as a Cercospora. *Cercospora personata* is considered as synonymous with *S. arachidis* and *C. arachidis;* whereas, it is stated that *C. arachidis* var. *macrospora* may possibly be a different species.

Miss Wakefield (25, p. 299) in 1921, and Mason (16) in 1928, list *Septogloeum arachidis* as a synonym of *Cercospora personata.*

Ciferri (8), in 1925, expressed the opinion that possibly there was a Cercospora other than *C. personata* attacking peanuts in Dominica.

Solheim and Stevens (24, pp. 368–369) include much detail in their description of *Cercospora personata,* which is evidently the same fungus as that of Ellis and Everhart. *Cladosporium personatum* and *C. arachidis* are given as synonyms, but no mention is made of *Septogloeum arachidis, C. arachidis* var. *macrospora,* or *C. arachidicola.*

Curzi (9) considers *Cercospora arachidis* and *Septogloeum arachidis* as synonymous with *C. personata* but expresses a doubt regarding *C. arachidis* var. *macrospora* in the statement "*Essa e stata descritta concaratteri corrispondenti pui a una specie distincta che a una varieta.*"

Saccardo (21) lists *Cercospora personata* and *Septogloeum arachidis* and cites *Cladosporium personatum* as a synonym of the former. No reference is made to *Cercospora arachidicola* and *C. arachidis* nor to the variety of the latter described by Maffei.

MATERIALS AND METHODS

Material for study included leaves from field-grown as well as leaves from artificially inoculated plants grown in the greenhouse. Material for

microscopical examination was fixed in Flemming's weaker solution or in a solution of picric and sulphurous acids in 75 per cent alcohol. Microtome sections were cut 6 to 8 μ in thickness and stained with Heidenhain's Iron-alum Haematoxylin.

In studying some of the herbarium specimens blocks of the leaves were soaked in a solution of potassium hydroxide for 24 hours, thoroughly washed, and fixed in Flemming's weaker solution.

Artificial inoculations were successfully made in a greenhouse with plants kept in a moist chamber. These chambers were frames covered with water-proof Cellophane or with Cell-o-glass. It was necessary to keep the plants covered in the greenhouse in order to obtain abundant infection and sporulation of the fungus.

A COMPARISON OF HERBARIUM SPECIMENS OF LEAF SPOTS OF THE PEANUT

The examination of herbarium specimens aided greatly in clarifying the last doubtful points regarding the nomenclature of the 2 fungi attacking the leaves of *Arachis hypogaea* L.

The following specimens have been examined:[4]

Ravenel, Fungi Caroliniani, Fasc. III, 1855.

85. *Cladosporium personatum* Berk. et Curt. foliis Arachidis hypogeae. Ex. Alabama quoque misit Beaumont foliis Cassiae occidentalis.

Ellis and Everhart, North American Fungi, Second Series.

2480. *Cercospora personata* (B. & C.) E. & E. Starkville, Mississippi, Oct. 1888. Prof. Tracy.

M. Raciborski, Cryptogammae Parisiticae, Insula Java Lectae.

Nr. 50. *Septogloeum arachidis* Rac. In foliis Arachi hypogaei. Tegal Leg. Raciborski.

Cercospora arachidicola Hori.

Collected by S. Hori Sept. 19, 1918. Matsudo, Prov. Schimosa.

Cercospora personata (B. & C.) E. & E.

J. C. Hopkins, Southern Rhodesia M. R. 1619. Amani, Tanganyika.

Cercospora personata (B. & C.) G. B. Wallace, Tanganyika. No. 1161.

Cercospora personata (B. & C.) E. & E. F. Eyles, Southern Rhodesia. Nos. 4987 and 5001.

Cercospora personata (B. & C.) E. & E. G. H. Jones, Nigeria (West Africa).

Microtome sections of the first 3 specimens were prepared and examined. Ravenel's collection described by Berkeley was found on examination to

[4] The writer is grateful to the following for the opportunity to examine herbarium specimens: Dr. C. L. Shear, Bureau of Plant Industry, Washington, D. C., and to Dr. S. F. Ashby, Imperial Mycological Institute, Kew, Surrey, England.

have conidiophores developing on definite stromata, produced hypophyllously. There was much intercellular mycelium sending many branched haustoria into the cells of the spongy mesophyll and palisade tissues. No conidia were observed either in sections or in mounts from the spots. However, an examination of a specimen of a similar leaf spot (*Cladosporium personatum* B. & C. var. *occidentalis*) on *Cassia occidentalis* showed many short obclavate or cylindrical conidia. This point, together with the internal mycelium, haustoria, and hypophyllous conidiophores of Ravenel's type specimen on peanut leaves, shows that Berkeley examined the same leaf spot as that studied later by Raciborski, Zimmermann, Ellis and Everhart, *et al.*

The collection (N. A. F. No. 2,480), determined by Ellis and Everhart as *Cercospora personata,* and Raciborski's specimen of *Septogloeum arachidis* were both found to be the same as Ravenel's collection described by Berkeley. The large stromatic conidiophore clusters and the haustoria are the same in all 3 specimens. (Figs. 1, G; 2, A).

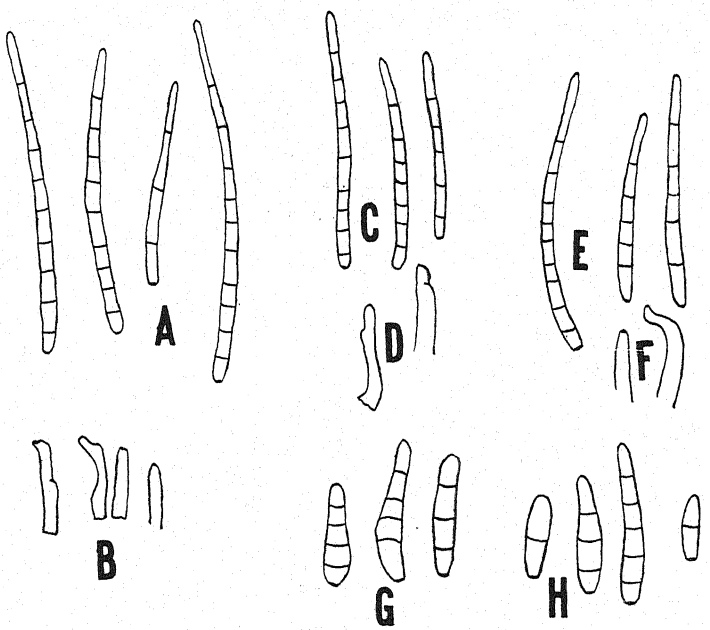


FIG. 1. Conidia and conidiophores. ×540. A–B. *Cercospora arachidicola* from fresh material collected in Georgia. C–D. *C. arachidicola,* type specimen collected by Hori in Japan. E–F. *C. arachidicola* collected by Eyles in South Rhodesia. G. *C. personata* N. A. F. No. 2480 identified by Ellis and Everhart. H. *C. personata* from material collected in Nigeria.

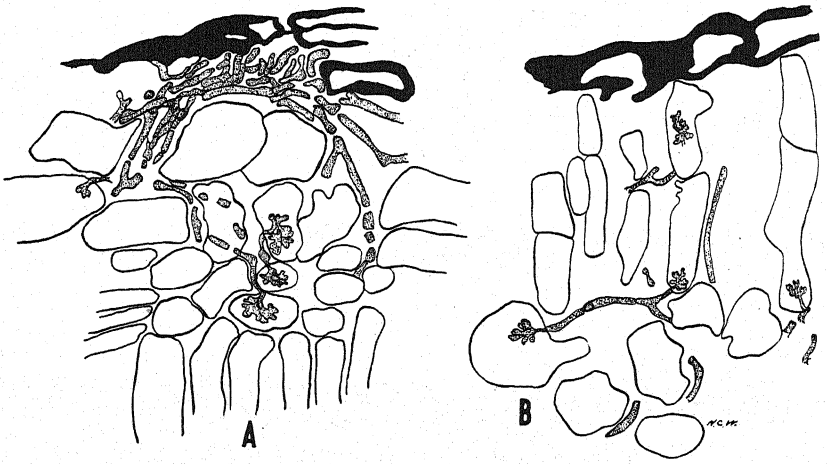


FIG. 2. *Cercospora personata.* ×493. A. Mycelium and haustoria from specimen classified as *Septogloeum arachidis* by Raciborski. B. Haustoria and internal mycelium from material collected in Georgia.

Hori's specimen of *Cercospora arachidicola* was found to have long, slender, obclavate conidia. The halos about the spots are distinct (Fig. 1, C–D).

The specimens of *Cercospora personata* collected by J. C. Hopkins in South Rhodesia were found to have long, slender, obclavate conidia. The same is true of the collections by Eyles from the vicinity of Salisbury in South Rhodesia (Fig. 1, E–F). The specimens collected by Wallace in Tanganyika and those of Jones in Nigeria were found to have short, thick, abruptly obclavate or cylindrical conidia. (Fig. 1, H). The conidiophore tufts on the lower surface of the leaf are concentrically arranged in many of the spots. The 2 latter are the same fungus as the type specimens of *C. personata, Cladosporium personatum,* and *Septogloeum arachidis;* whereas, the first 3 are the same as the species described by Hori as *Cercospora arachidicola* and probably by Maffei as *C. arachidis* var. *macrospora.* They are also the same as a leaf spot found in Georgia.

COMPARATIVE DESCRIPTIONS OF TWO PEANUT LEAF SPOTS AND THEIR RESPECTIVE CAUSAL FUNGI

Both microscopically and macroscopically the 2 leaf spots attacking peanut leaves are quite easily distinguished, especially when the spots become older.

Cercospora arachidicola hori. Syn. *C. arachidis* P. Henn. var. *macrospora* Maff.

Cercospora arachidicola produces irregularly circular, often confluent spots varying in size from 1 mm. to 1 cm. in diameter and surrounded by

a bright yellow halo, blending into the green of the leaf. Initial spots are yellowish specks. The color of the older spots varies from dark brown to almost black on the upper surface of the leaf but is usually a lighter brown color on the under surface. The yellow border is less pronounced on the lower than on the upper surface. (Fig. 3, A). Elliptical spots are often found on the stems and leaf petioles later in the season.

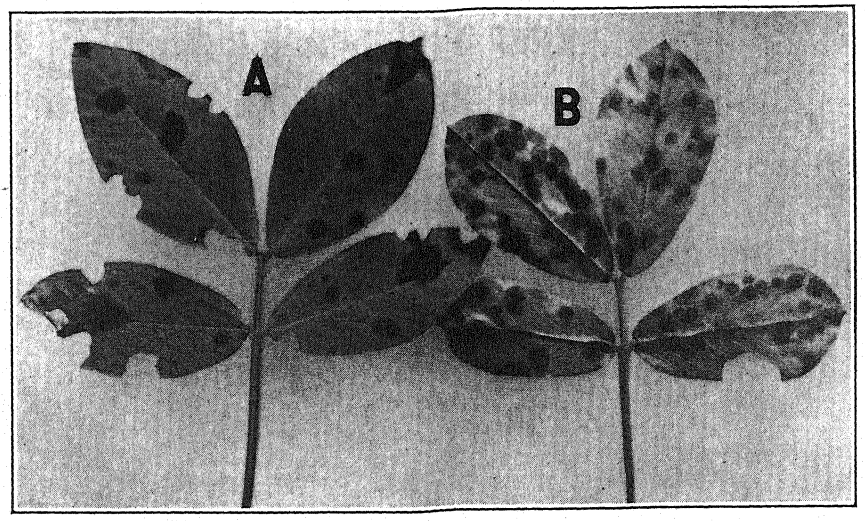


FIG. 3. A. Upper surface of peanut leaf showing large irregular spots of *Cercospora arachidicola*. B. Lower surface of peanut leaf showing the raised round spots of *C. personata*.

The mycelium is both internal and external. No haustoria have been noted. The mycelium is both intracellular and intercellular.

The conidiophores of *Cercospora arachidicola* are amphigenous. In young spots the conidiophores are produced almost exclusively on the upper surface of the leaf, but in older spots a few may sometimes be found on the lower surface. The conidiophores are produced in 3 different ways; first, they emerge directly through a stoma, forming a small fascicle of conidiophores; second, the mycelium grows out between epidermal cells or through a stoma and continues to develop under the cuticle, and from this mycelium fascicles of conidiophores are formed; third, conidiophores form from mycelium growing in epidermal cells that are later ruptured by the developing conidiophores. The bases of the conidiophore groups are usually somewhat stromatic, especially when mature. (Fig. 4).

The conidiophores are subgeniculate 21.6–40.5 × 3.2–5.4 μ, yellowish brown, continuous or 1- to 2-septate. The scars marking the point of at-

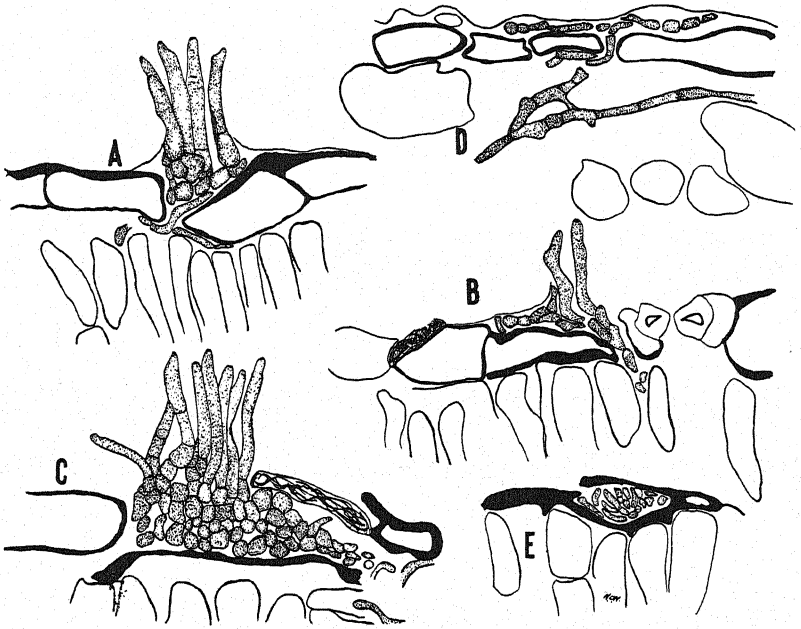


FIG. 4. *Cercospora arachidicola.* ×493. A–B. Conidiophores developing from mycelium beneath the cuticle on the upper surface of a leaf. C. Mature group of conidiophores showing the stromatic base. D. Mycelium growing beneath the cuticle on the lower surface of the leaf. E. Mycelium of a young conidiophore fascicle developing within an epidermal cell.

tachment of the conidia are plainly visible. The colorless, or pale yellowish to slightly olivaceous, obclavate conidia, measure 37.8–108 × 2.7–5.4 μ and are 4- to 12-septate, the usual number being 5 to 7. In dry weather the number of septations are fewer. (Fig. 1, A–B).

The conidia germinate in water and in slight accumulations of water on the surface of cover glasses placed over a van Tiegham cell. The manner in which they germinate depends on the amount of moisture present.

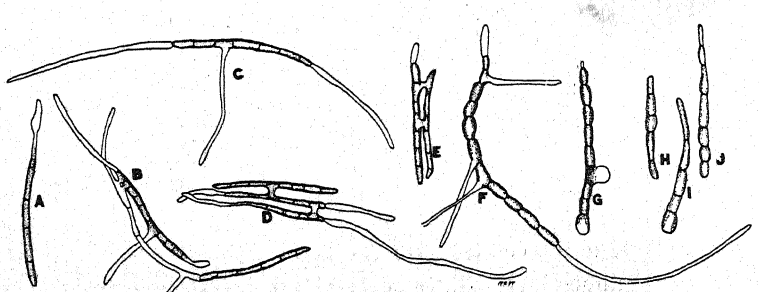


FIG. 5. Germinating conidia of *Cercospora arachidicola.* ×360. A–F. Germinated in heavy film of water on cover glass placed over water in a van Tiegham cell. G–J. Swelling of cells in a film of water too thin for ready germination.

Under favorable conditions long germ tubes develop in about 12 hours from any of the several cells of the conidium, often several tubes from a single conidium. With insufficient moisture the cells may swell without developing germ tubes (Fig. 5).

Spermogonia develop on fallen leaves late in September or early in October. At about the time the spermogonia begin to develop the internal mycelium becomes very thick walled.

In artificial culture the mycelium grows very slowly, forming a dense black mass. Conidia have not been observed in culture.

Cercospora personata (B. & C.) E. & E. Syn. *Cladosporium personatum* B. & C. *Septogloeum arachidis* Rac. *Cercospora arachidis* P. Henn.

Cercospora personata spots are circular and measure from 1 to 7 mm. in diameter. Halos are wanting or are a pale yellowish green on the lower surface. Bright yellow halos develop around the more mature spots on the upper surface of the leaf.

The mycelium is internal, no external mycelium having been observed. Branched haustoria develop in the palisade and spongy mesophyll cells (Fig. 2, B). Late in the season the mycelium becomes thick walled.

The conidiophores are amphigenous but more numerous on the lower surface, where the tufts are often arranged concentrically, especially in the larger spots. These can be seen easily with the naked eye. (Fig. 3, B). Elliptical spots often appear on the stems and on the leaf petioles.

The conidiophores measure 24.3–54 × 2.7–8.1 μ and are continuous, or 1- to 2-septate, subgeniculate or shouldered, the points of attachment of the conidia being plainly visible. The color is reddish brown and the apices are often hyaline. The conidiophores develop from loosely stromatic masses of mycelium that form beneath the epidermis, frequently in the spaces beneath the stomata. The epidermis is ruptured as the conidiophores emerge. The bases of the tufts become more densely stromatic as they mature. (Fig. 6, A, B).

The obclavate or cylindrical conidia measure 18–60 × 5.4–10.8 μ and are 1- to 7-septate. The larger conidia are usually rather abruptly attenuated at the apex. In some instances the shorter 1- to 3-septate conidia are clavate rather than obclavate. The color is a pale brown and in some cases almost olivaceous. The conidia are produced singly. (Fig. 6, C, D).

Spermogonia develop during the fall.

DISCUSSION

It is clear from the literature review that 2 leaf spots may occur wherever peanuts are grown. That this fact has been generally recognized

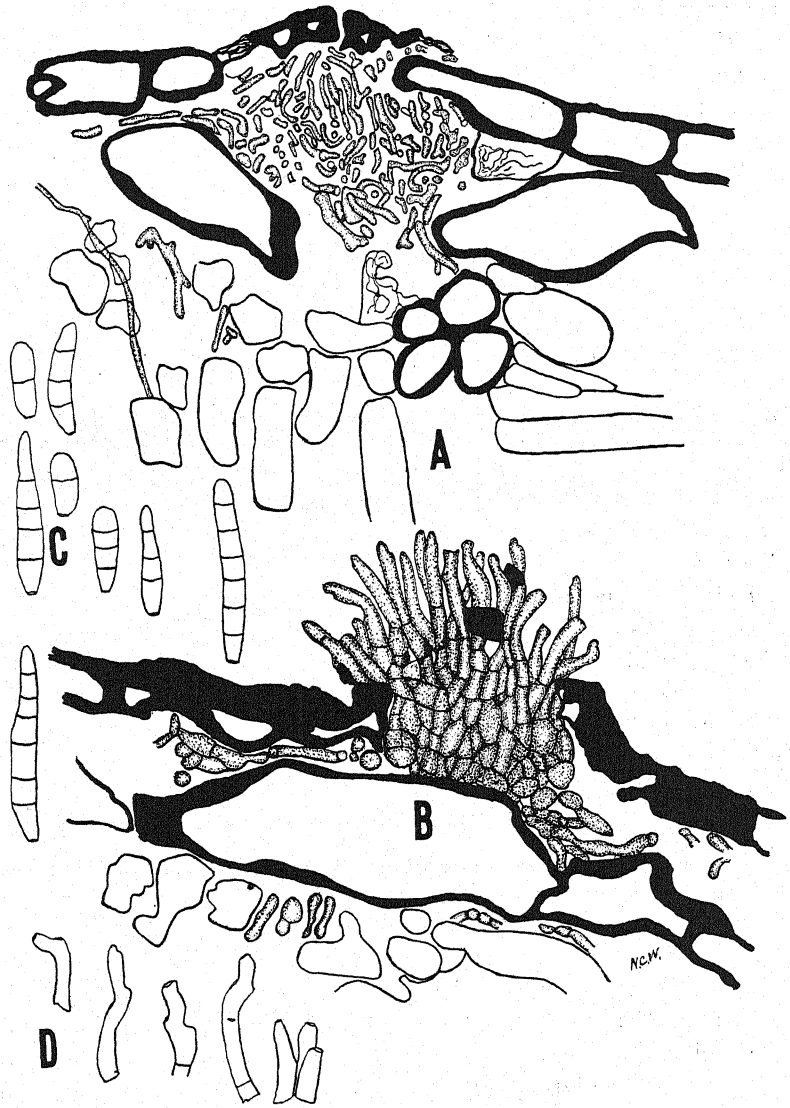


FIG. 6. *Cercospora personata.* × 540. A. Primordium of conidiophore tuft developing beneath a stomatal opening on the lower surface of a leaf. B. Mature conidiophore tuft showing the stromatic base. C. Conidia. D. Conidiophores.

without distinguishing the 2 has led to much confusion in the description and nomenclature of the causal fungi. Hori (13, p. 26) and Sawada (22) have described both leaf spots and their causal fungi and are the only writers who seem to have understood the confusion existing. Unfor-

tunately their descriptions are little known.[5] The purpose of this paper has been to direct the attention of pathologists and mycologists to the occurrence of 2 leaf spots on the peanut (*Arachis hypogaea* L.).

From the literature review and from observations at the Georgia Experiment Station it has been concluded tentatively that *Cercospora arachidicola* occurs rather consistently year after year, even when the weather is quite dry. *Cercospora personata* occurs at irregular intervals. In some seasons it is very severe, while in others few, if any, spots are to be found. In the 2 seasons in the last 5 years that *C. personata* has appeared at the Georgia Experiment Station it was undoubtedly the most destructive. More complete defoliation occurred in the years when *C. personata* was abundant. The conditions conducive to the development of *C. personata* are not well enough understood to attempt to explain why it appears only in certain years. Possibly wet seasons are more favorable to its development than the drier seasons.

SUMMARY

Two leaf spots have been found attacking the leaves of peanuts (*Arachis hypogaea*) in Georgia.

The nomenclature of the fungi attacking the leaves of peanuts is much confused.

A study of literature and a comparison of specimens from several collections have shown that there are often 2 leaf spots in nearly all peanut-growing areas, both sometimes appearing on the same leaf.

Cercospora personata (B. & C.) Ell. & Ev. has been generally accepted as the correct name of one of the fungi attacking the peanut. This fungus has abruptly obclavate or cylindrical conidia and the conidiophore tufts are concentrically arranged in the spots on the lower surface of the leaf.

Cercospora arachidicola Hori is probably the most common but is apparently the least destructive of the 2 fungi.

EXPERIMENT, GA.

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"BIG BUD," A VIRUS DISEASE OF THE TOMATO

GEOFFREY SAMUEL,[1] J. G. BALD,[2] AND C. M. EARDLEY[3]

(Accepted for publication August 31, 1932)

"Big bud" disease of the tomato was first described by Cobb (2) in New South Wales, under the name "rosette." The disease is now known by tomato growers in different localities in Australia under the names big bud, rosette, "blue top," or "bunchy top." For reasons given below, it has been considered advisable to adopt the name big bud as being the most generally satisfactory common name.

Since Cobb's first description of the disease, only short notes on it, with or without an illustration, have appeared in general articles on tomato diseases in Australian agricultural journals (3, 5, 9, 10, 12). In all the notes that have appeared to date, it is stated that the cause of the disease is not known, although in the New South Wales Farmer's Handbook (9) it is said that "the available evidence suggests that it is a disease of the virus type."

The experiments reported in the present paper confirm this suggestion and show that the disease can be transmitted by budding and grafting. No insect vectors have yet been discovered. The following account is presented mainly with the idea of calling attention to morphological and anatomical changes, which differ from those associated with any other virus disease.

NAME OF THE DISEASE

The name rosette, used by Cobb, has priority as far as date of publication is concerned, and it would have been retained if there had been no objections to it. These may be stated as follows:

(1) The name "tomato rosette" has since been used for other diseases. Selby (11) used the terms "potato rosette" and tomato rosette in the early part of the century for a disease that he attributed to *Rhizoctonia solani,* but his conclusions have not been generally accepted. Chupp (1) gives the name rosette for a tomato disease of unknown cause investigated by Orton and McKinney in Pennsylvania, but the report of this work is not available to us. From the description of symptoms given by Chupp, however, the disease is evidently different from the one dealt with in this paper.

(2) Although it is admitted that the final effect of the Australian disease is to give affected plants a dense, tufted habit, well described by the

[1] Plant Pathologist, Waite Agricultural Research Institute, Glen Osmond, South Australia.

[2] Assistant Plant Pathologist, Division of Plant Industry, Commonwealth Council for Scientific and Industrial Research.

[3] Graduate Student, University of Adelaide, South Australia.

term rosette, this is not evident until a comparatively late stage. The most pronounced early symptom is an enlarged bladder-like calyx; and it seems advisable on general grounds to draw the attention of growers to early symptoms in preference to late. (Froggatt (5) even considered the early stage to be a separate disease, for which he proposed the name "Sheath-calyx.")

(3) It will become apparent from the description given below that a number of the symptoms are not paralleled in the rosette diseases of the virus type (peach rosette, wheat rosette, peanut or groundnut rosette) already described.

For these reasons the name big bud is proposed in place of rosette. It has the merit of being in fairly general use already, of drawing attention to the earliest symptom of the disease, and of avoiding confusion with the other tomato-rosette conditions. It also leaves the term rosette free in case one of these diseases is later found to be a definite entity requiring a name of this type. One disadvantage of the name, though not considered a serious one, is the fact that it has already been used for a disease of black currants in England caused by mites, which is often associated with the virus disease "reversion." There appears to be no connection between the big-bud conditions caused by the mites and the virus disease, however.

R. J. Noble and D. B. Adam, plant pathologists in New South Wales and Victoria, respectively, have been consulted, and also favor the adoption of the name big bud. The name bunchy top should be avoided and reserved for the disease described by McClean (8) from South Africa.

OCCURRENCE

As far as is known, tomato big bud does not occur in any other country than Australia, where it is found to some extent in all the States, but most frequently in New South Wales and Victoria. Usually only a very small percentage of plants are affected, and these occur scattered at random in the field. In South Australia, over a period of 10 years, no case has been seen where more than 5 per cent of plants have been affected, and the number is usually much smaller. In Victoria, Strickland (12) speaks of the results from manurial plots in 1930 being spoiled by the disease, which occurred to the extent of 10 per cent. In New South Wales, considerably higher proportions have been recorded at times. R. J. Noble, in a letter, speaks of cases where 50 to 100 per cent infection has been observed.

The prevalence of the disease varies from season to season, probably depending largely upon the abundance of some as yet undiscovered insect vector. It is predominantly a late-season disease, appearing in January or February, the midsummer and late summer months.

Plants affected with big bud may also become infected with spotted wilt or mosaic, and occasionally plants already infected with these diseases have been noticed to become affected with big bud.

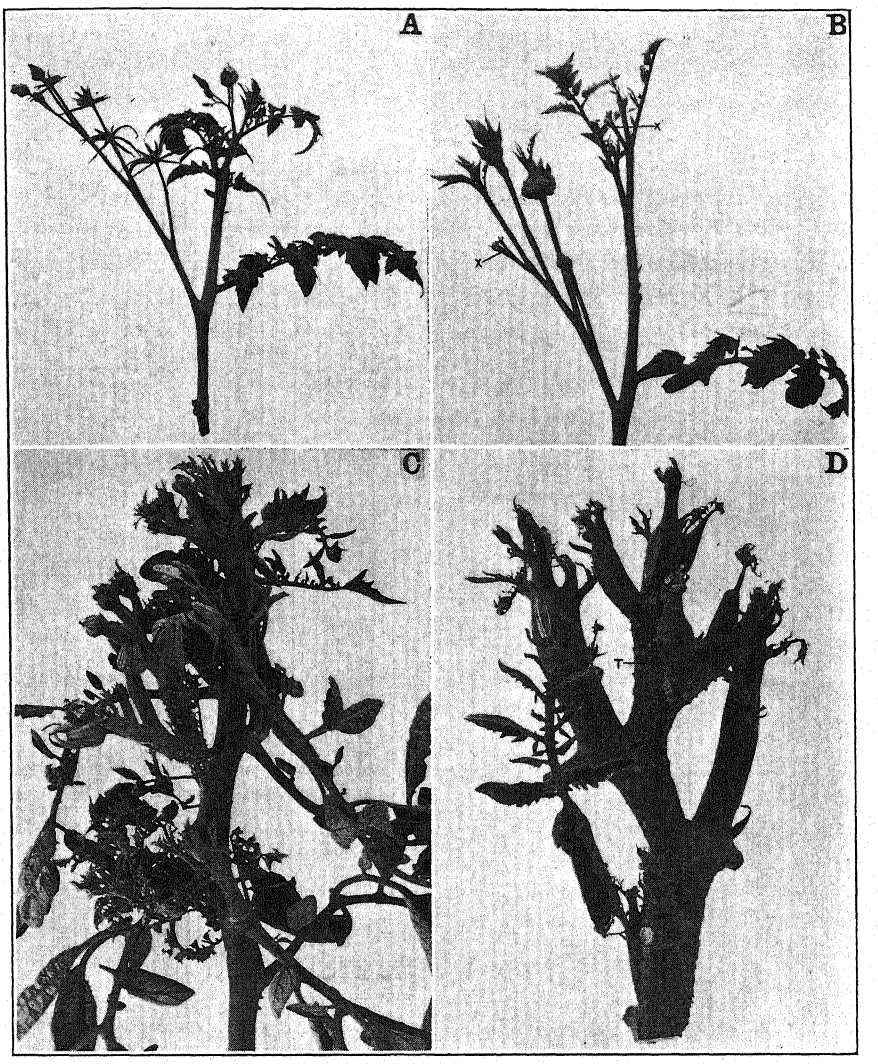


FIG. 1. A. Shoot of Dwarf Champion tomato with early stage of big bud. The flowers on the lowest truss were just opening when the virus invaded the top of the plants. B. Shoot of infected plant about 5 weeks after symptoms first visible. Note reduction of flower trusses to papillate branches at X. Both A and B from plants budded in glasshouse. C. Fairly late stage of big bud on a pruned Sensation plant from the field. D. Late stage on a pruned Marglobe plant from the field. Note that the branch T is morphologically a fruit truss.

SYMPTOMS

Plants affected with big-bud disease are characterized by some remarkable teratological growth changes, particularly in the flowering parts.

The Shoot. The first indication of infection appears at the tips of actively growing shoots. The youngest fruit truss, instead of becoming recurved as in normal plants, assumes an upright position (Fig. 1, A). The buds on the truss also point in a vertical direction, the calyx segments remain united almost to the tips, and the whole calyx enlarges to a form like a bladder with a toothed opening at the top. On pruned plants in the field the main shoot soon ceases growth, and the young leaves at the growing point fail to develop normally. A purple coloring due to the development of anthocyanin is often particularly marked along the veins of the bladdery calyces, on the under sides of the young leaves and on the youngest portion of the stem.

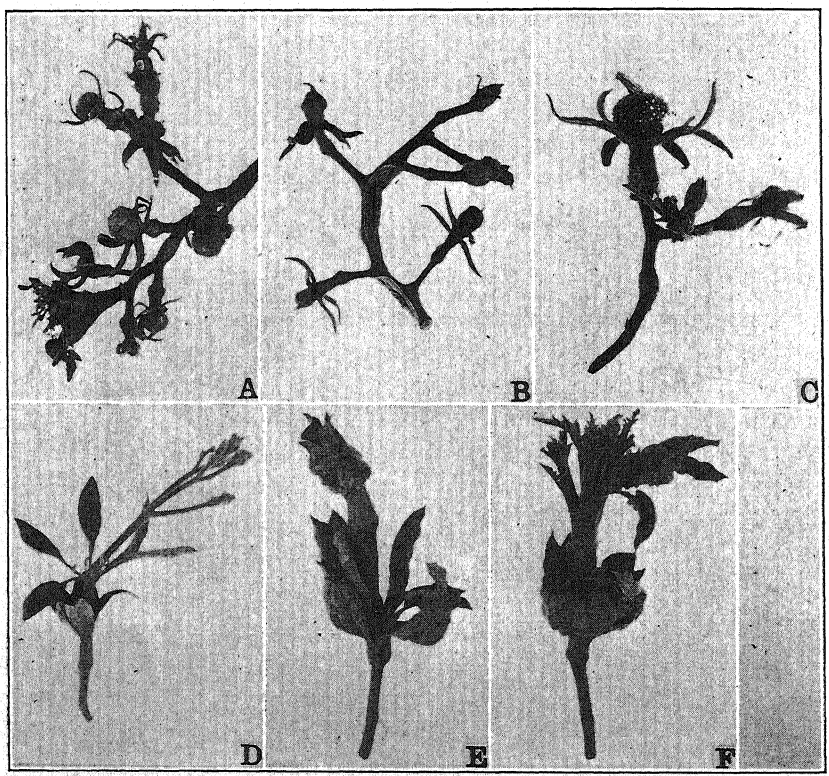


FIG. 2. A–C. Effect of the big-bud virus when it invades fruit trusses that have just set. Note arrest in growth of fruit, bursting of hardened pericarp exposing aborted ovules, thickening of pedicels commencing at abscis layers, adventitious shoots on pedicels, and longitudinal splits exposing inner tissues. D–F. Different types of floral proliferation on tomato × currant-tomato crossbreds infected with big bud.

After a short time the axillary buds grow out, forming shoots affected in the same way as the main shoot. Simultaneously, there is a gradual thickening of the stems of the affected parts (Fig. 1, C) due to the formation of an abnormal tissue, described later, in close association with the internal phloem. In cases where the growth of the terminal buds completely ceases, the thickening of the stems may become very marked, and a diameter of $1\frac{1}{2}$ to $2\frac{1}{2}$ cm. be attained, even in what is morphologically a fruit truss. (Fig. 1, D.) Adventitious root initials break through the epidermis, and longitudinal splits may appear, laying bare the internal tissues in scars of characteristic form. (Fig. 1, D, and Fig. 2, B.)

On infected plants that are unpruned there appears to be more outlet for the increased growth activity in the development of numerous lateral shoots. The axillary buds of these lateral shoots grow out in turn, instead of remaining dormant, and the flowers proliferate as described below, so that the infected plant finally acquires a very tufted, rosette habit of growth. Partially pruned plants with a number of laterals already in this late stage are shown in figure 3, A and B.

In the greenhouse infected plants were observed to grow more rapidly than the uninfected at first, but after a few weeks they gradually ceased growth at the tips and acquired small purplish leaves. (Fig. 1, B.)

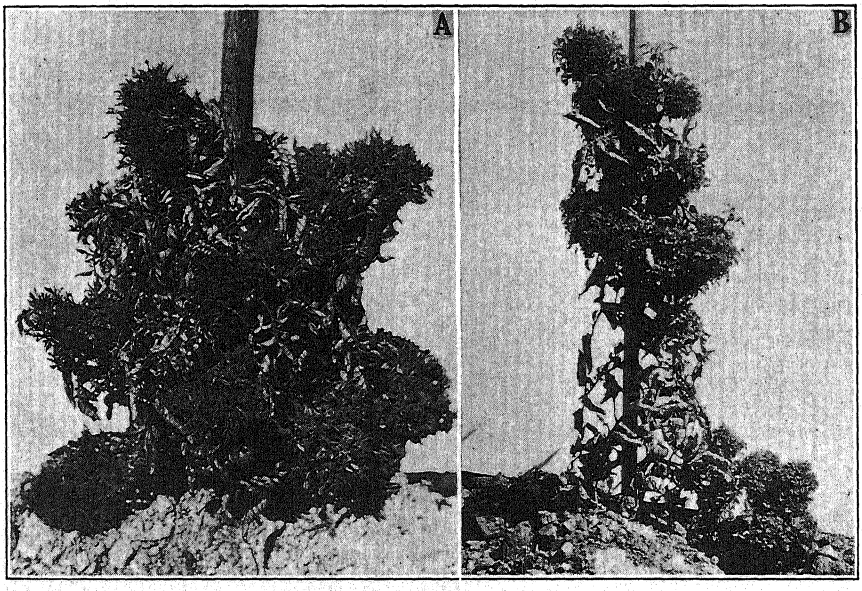


FIG. 3. Late stage of big bud on partially pruned field plants. A. At Bendigo, Victoria. B. On tomato × currant-tomato crossbred, Waite Institute, South Australia.

The Leaves. The first effect of the virus on young developing leaves may be to stimulate the formation of more small lateral leaflets from the main and secondary petioles. There may also be some upward rolling of the leaf margins. After some time the youngest leaves become yellowish green in color, particularly at the margins, the lateral leaflets are often much reduced (Fig. 1, B–D), and there is usually a purpling underneath due to the formation of anthocyanin in the cells of the lower epidermis. As the disease progresses, smaller and smaller leaves are formed, until in the dense rosettes that are finally produced the leaves may be only a centimeter or 2 long.

The Flower. Flowers that are fully formed at the time the virus invades the truss become erected from their recurved position but undergo little change beyond a slight virescence of the petals, appearing first along the midribs (Fig. 1, A). In younger flowers, just opening at this time, the corolla may become either stunted or enlarged and completely virescent. In both cases, there is a cessation of further development of anthers and ovary.

In buds at the end of the first infected truss and in all buds formed on subsequent trusses (Fig. 1, A) the sepals remain united and enlarge, so that the calyx swells up to the bladdery form previously described. Buds such as these never develop to normal flowers, and the central parts are either completely arrested in growth or proliferate in various ways, as follows.

There may be marked phyllody of the corolla, which frequently develops to a whorl of simple green petiolate leaves 1 to 3 cm. in length (Fig. 2, D–F). The insertion of this whorl of corolla leaves may remain close to the base of the calyx, the leaves scarcely or not projecting, or it may occasionally be carried up by an extension of the floral axis as much as 2 cm. above the calyx insertion (Fig. 2, D).

The anthers are usually inserted just above the foliaceous petals; they are pale in color and gradually die from the tip downwards without dehiscing. Owing to the thickness of the proliferated axis of the flower they are not syngenesious, as in normal flowers, but are inserted singly between the corolla leaves (Fig. 2, D). Occasionally single anthers are carried partially up the proliferated central axis.

The gynoecium is frequently carried up some 1 to 2 cm. on the proliferation of the central axis of the flower. In these cases, there is usually a distinct phyllody of the carpels, the individual leaves being either completely united to their apices or at least partially united by their margins and petioles (Fig. 2, E and F). In the former case, a transverse section of the gynoecium shows placental ridges bearing rudimentary ovules, in cavities with thin foliaceous outer walls. In the latter case, where there

is further development of the carpellary leaves, the extended axis of the flower gives rise to a system of short, thick-branched shoots some 1 to 2 cm. long (Fig. 2, F) with a characteristic dichotomous forking at the apices. Sometimes, however, the proliferating branches terminate in a second series of affected flower buds (Fig. 2, D). The proliferation of the central axis of the flower to a number of short-branched shoots may also occur without there being any sign of carpellary leaves developed.

The short shoots with dichotomous branching at the apices, which usually arise from the proliferated floral axis, bear minute colorless papillae at their tips (Fig. 4, A), with a few small leaf rudiments intermingled. They

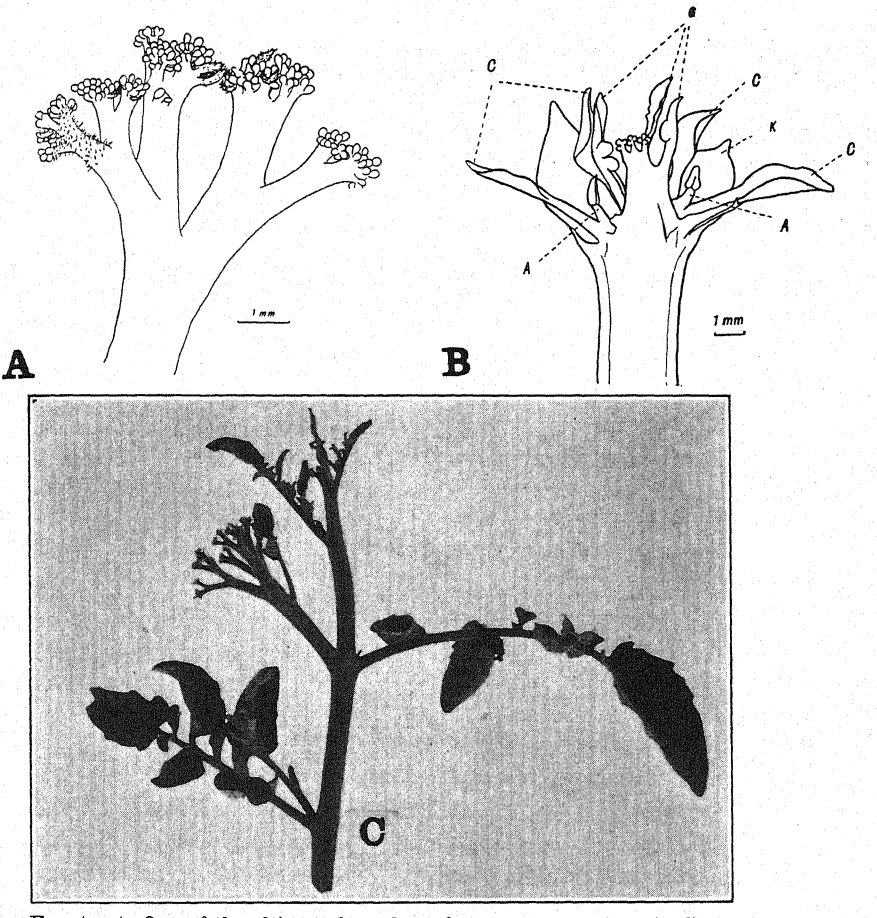


FIG. 4. A. One of the ultimate branches of a structure such as in C, showing papillae intermingled with leaf rudiments. B. Longitudinal section of a flower in which in addition to virescent petals and foliar carpels is a central structure with papillae. C. Reduction of flowering truss to a dichotomously branched structure with papillae at the tips, on a tomato × currant-tomato crossbred infected with big bud.

are usually strongly colored with anthocyanin. From occasional cases, such as that shown in Figure 4, B, where these papillae appear at the summit of a single short structure in the position of the gynoecium, the papillae appear to be homologous with ovules. There is no structural differentiation within them, however, and they later turn brown and die.

In later stages of the disease, the characteristic system of dichotomously branched shoots terminating in papillae frequently arises directly from the stem (Fig. 4, C) in such a position as to indicate that it represents a complete flower truss. In these cases it would appear as if the intermediate stage of flower formation had been suppressed and the final end of proliferation to this remarkably changed gynoecial structure attained directly.

The Fruit. Fruit already set when invaded by the virus is immediately arrested in growth (Fig. 2, A–C). In very young fruit there is sometimes a slow subsequent growth of the placental tissues that bursts the thin ovary walls and exposes the aborted ovules (Fig. 2, A). The latter may grow for a little while to greenish papillae (devoid of internal structure) projecting from the exposed placenta (Fig. 2, C). Meanwhile, the usual thickening of the stems characteristic of the disease appears on the younger parts of the truss, becoming most pronounced first at the abscis layer below each fruit (Fig. 2, A–C). Adventitious shoots may arise later from several places on the truss and grow and branch profusely in the usual way until they finally form a rosette of small leafy shoots. The adventitious shoots may arise not only from any part of the pedicels but from the aborted placental tissue, or even from between the arrested ovary and the calyx, in the position of the corolla.

Fruit that was well developed but still green at the time of infection becomes hard and tough and colors extremely slowly or not at all. In the younger fruit there may be a necrosis in the centers of the young embryos, which are embedded in a firm parenchymatous tissue with thick cellulose walls instead of in a tissue soon becoming gelatinous, as in normal fruit.

The Roots. No pathological changes have been observed in the roots of plants affected with big-bud disease.

ANATOMICAL CHANGES

The anatomical changes associated with the abnormal flower parts will not be dealt with in this paper. Here it is desired to call attention only to a very characteristic tissue that develops in association with the internal phloem in affected parts of the stem. In very much thickened stems, such as those in figure 1, C and D, this tissue is easily visible to the naked eye. When the stem is cut across it is seen as a band about 1 to 2 mm. across, internal to the wood, having a somewhat greenish, water-soaked appearance, owing to the close packing of the cells of which it is composed.

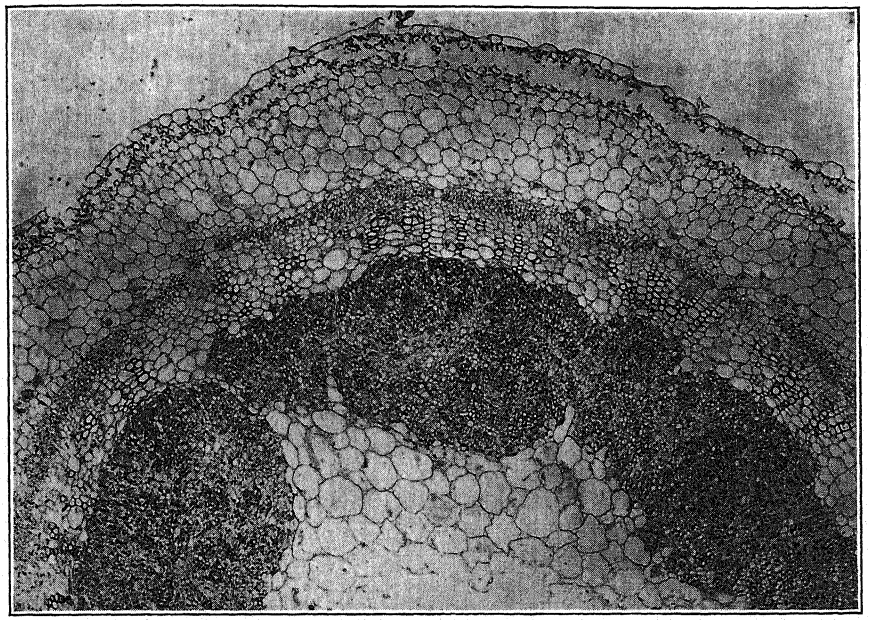


FIG. 5. Photomicrograph of a transverse section of a pedicel below an enlarged calyx. Note great development of adventitious tissue in place of internal phloem.

A transverse section from the pedicel below a bladder-shaped calyx is illustrated in figure 5. The great development of internal phloem tissue characteristic of the disease is immediately evident. The tissue is unlike normal phloem, however, in that the great bulk of it consists of small cells with prominent nuclei very similar to companion cells, that sieve tubes are rare, and that isolated tracheids are also found within it.

The tissue is found in all parts of the stem formed subsequent to infection by the virus. Arising first in scattered groups, resulting from the division of isolated pith cells, it soon increases and forms a continuous band. It is present also in considerable amount in the pedicels of affected fruit trusses and to a less extent in the petioles and midveins of the leaves. Wherever much of this tissue has been formed considerable amounts of starch are present in the pith.

No intracellular inclusions of the type described for certain mosaic diseases have been noted.

TRANSMISSION TESTS

Mechanical Transmission. No cases of successful mechanical inoculation of tomato big bud have yet been obtained. Freshly expressed juice from diseased field plants was used for inoculation to healthy tomato plants,

varying from seedlings to plants 15 in. or more in height. Inoculation was by rubbing the surfaces of a number of leaves, by needle scratching, or by injections of juice with a hypodermic syringe. The plants were kept from 5 to 9 weeks after inoculation, but all the experiments, which were done at various times, gave negative results. A number are summarized in table 1.

Budding and Grafting. Budding and grafting experiments, in which buds and scions from field plants diseased with big bud were used, have given successful transmission in every case in which the bud or scion remained alive. Transmission occurred even when the bud itself died and only a small pad of cortical tissue from the diseased plant lived and formed organic union. No transmission occurred in any case when the buds or scions died completely except in 3 cases in which grafted plants contracted the disease in spite of death of the scions. The scions on these grafted plants died very slowly, however, and it is possible that a small portion of

TABLE 1.—*Summary of transmission tests with big-bud disease of tomato*[a]

Date	Method of inoculation	Plants inoculated	Plants infected	Incubation period (days)	Notes
8.3.30	Rubbing +	3 tomato	0	.....	Discarded after 38 days
	Needle	3 tobacco	0	.....	
	Scratching	3 *Nic. glutinosa*	0	.....	
13.3.30	Rubbing +	5 tomato	0	.....	Discarded after 33 days
	Needle	5 tobacco	0	.....	" " 42 "
	Scratching	3 *Nic. glutinosa*	0	.....	" " " "
—.1.32	Rubbing	10 tomato	0	.....	Discarded after fruit set
17.3.32	Rubbing	24 tomato	0	.....	" " 65 days
17.3.32	Hypodermic	24 tomato	0	.....	" " " "
1.2.32	Grafting	10 field tomatoes	1	32	9 grafts failed
13.2.32	Budding	4 field tomatoes	4	31, 37, 37, 43	
18.2.32	Budding	16 field tomatoes	16	28–36	
8.3.32	Grafting	10 tomato	3	47–84	10 grafts but none took, 3 cases of transmission
8.3.32	Budding	15 tomato	15	47–56	Repotted when pot-bound
		4 tobacco	0		6 other buds failed
		4 *Nic. glutinosa*	0		2 other buds failed
17.3.32	Budding	6 tomato	6	56	Repotted when pot-bound

[a] All plants were kept in an insect-proof greenhouse except where it is definitely stated that field tomatoes were inoculated. Controls in the greenhouse and in the field, budded with healthy buds, remained healthy. There was no further natural spread of the disease in the surrounding field plants at the time the successful transmissions were obtained by budding and grafting.

tissue in a favorable position formed organic union during the first week or 2, but later died with the rest of the scion. The budding and grafting experiments are also summarized in table 1.

The incubation period varied from 28 to 56 days. The longer incubation periods were on older plants in the field or on plants that became rather potbound in the greenhouse. Many of the greenhouse tests were done on plants in 6-in. pots before it was realized that the incubation period was always long, and after some 3 to 4 weeks, when growth was obviously slowing up because of the small pots, the plants were transplanted to 8-in. pots and later developed the disease. It is considered that the average incubation period in plants kept growing under good conditions will be found to be somewhere near the minimum obtained in these experiments.

HOSTS OTHER THAN TOMATO

Plants of *Solanum nigrum* have been found near the tomato plots, almost certainly affected with the big-bud disease. Besides reduction in size of the leaves, and the presence of numerous axillary shoots, the flowering trusses were replaced by short branches with terminal papillae of the type described for the tomato disease. An adventitious internal phloem tissue similar to that found in tomato big bud was also present. In attempts to transmit the disease by budding to tomatoes, all the buds unfortunately died.

Four tobacco plants and 4 *Nicotiana glutinosa* plants were successfully budded with diseased tomato buds. No transmission to these plants took place, however, and flowering, which occurred some 14 to 15 weeks after budding, was quite normal in both species, the inserted diseased buds being still alive and a few of them having grown considerably.

DISCUSSION

The disease that presents the closest parallel to tomato big bud as regards effect on the host plant is undoubtedly cranberry false blossom, transmitted by the jassid *Euscelis striatulus* (4). A stimulation to the growth of axillary shoots, erection of the normally recurved pedicels, enlargement of the calyx lobes, virescence of the petals, abnormalities in stamens and ovaries, and frequent floral proliferation are all characters in common between the two diseases. Teratological changes proceed somewhat further in the tomato disease, however, in adhesion of the enlarged sepals, more marked changes in proliferation of the ovary, and particularly in the formation of the branches bearing papillae (Fig. 4).

Less marked floral changes, including virescence and proliferation, have been described for aster yellows and alloiophylly of anemones (6), but in these cases they are not invariably associated with the diseases. And, of

course, a stimulation to the growth of lateral shoots and a general upright habit of growth are characteristic of a number of virus diseases. One such disease, witches' broom of potatoes, has been transmitted to tomatoes (13). On the latter host, it has the following symptoms in common with tomato big bud: (1) the shooting of axillary buds, (2) marked dwarfing of the leaves at the growing points, (3) yellowing of the margins of the young leaves, and (4) purpling of the veins on the under sides of the leaves. That the diseases are distinct, however, is certain from the description of witches' broom on tomato given by Young and Morris (13). They state that their plants "bloomed profusely, and produced many small fruits; the buds and flowers developed normally." Their illustrations show flower trusses normally recurved, and there is a spindliness of infected stems rather than the thickening characteristic of tomato big bud.

The only parallel to the adventitious tissue that arises in association with the internal phloem in tomato big bud seems to be the phloem gall of Fiji disease of sugar-cane (7). This, however, is localized, so that numerous discrete galls are produced, whereas in tomato big bud the tissue soon forms a continuous thick band in all the new growth produced after infection, and the external phloem is not affected.

SUMMARY

Big-bud disease of tomato, originally named tomato rosette by Cobb, occurs in all Australian States, but most frequently in New South Wales and Victoria.

Abnormalities in the floral parts, involving reversal of the normal geotropic response, and various forms of over-growth, adhesion, virescence and proliferation are the most characteristic symptoms. The shooting of axillary buds and the formation of dwarfed leaves lead to a very tufted habit of growth in the later stages.

The stems of affected plants are somewhat thicker than those of normal plants, due to the formation of an abnormal tissue in association with the internal phloem.

The disease could not be transmitted by mechanical inoculation, but readily by budding and grafting, the shortest incubation period being 28 days.

The insect vectors of the disease have not yet been discovered.

Some field plants of *Solanum nigrum* showed symptoms similar to those of diseased tomatoes.

Buds from big-bud diseased tomatoes did not transmit the disease to tobacco or *Nicotiana glutinosa*.

WAITE AGRICULTURAL RESEARCH INSTITUTE,
UNIVERSITY OF ADELAIDE,
SOUTH AUSTRALIA.

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"SUSPECTED MOSAIC" OF THE STRAWBERRY[1]

E. F. GUBA

(Accepted for publication September 19, 1932)

In view of the interest being shown in this chlorotic leaf condition of the strawberry (Fig. 1), it may be timely to offer a brief account of the writer's observations in Massachusetts and of his efforts to determine the nature of this disease. Some of the information obtained confirms the results of other workers and is offered to support certain opinions as to the nature of the trouble.

This strawberry disease has already been studied by Berkeley (1, 2), Plakidas[2] (5), and Clark (3). Berkeley, who called it "suspected mosaic," was unable to produce the disease artificially. He found it to be seed-borne and systemic. According to him, it is confined to new seedling varieties, and does not spread in the field from one variety to another. The illustration accompanying Berkeley's article shows the chlorotic character of the disease and the pronounced dwarfing of a diseased plant. In Ontario, the symptoms appear with the unfolding of the leaves in the spring, disappear with the hot weather of early summer, and reappear again the following spring. These observations led Berkeley to entertain the feeling that the trouble was of genetic origin. Clark, who called the disease "non-infectious chlorosis," considered its connection with a virus of the mosaic type rather unlikely, because (a) the chlorotic leaves are not crinkled, (b) most, but not all, of the seedlings of chlorotic plants are chlorotic, and (c) all degrees of chlorosis prevail from apparently normal plants to those almost completely devoid of chlorophyll. The crinkling of the leaves, or a variation in the intensity of the symptoms, should not, however, argue more strongly for a genetic than a virus hypothesis. The crinkled condition of some of the foliage is quite characteristic of the disease (Fig. 1, A). According to Clark, it seemed probable that the condition was hereditary and was caused originally by a gene mutation, although further convincing evidence seemed desirable. Plakidas, like Berkeley, failed to produce the disease artificially with sap transfers, insects, or by reciprocally grafting diseased and healthy strawberry runners. He noted that a percentage of seed-propagated plants from chlorotic parents developed the disease the second year. Thus, very good published evidence makes doubtful the infectious nature of the trouble, but neither has the hypothesis of an hereditary condition been entirely convincing.

[1] Massachusetts Agricultural Experiment Station Contribution No. 152.

[2] Plakidas, A. G. Report on strawberry virus project. U. S. Dept. Agr., Bur. Plant Indust., Plant Dis. Rptr. 13: 129–131. 1929.

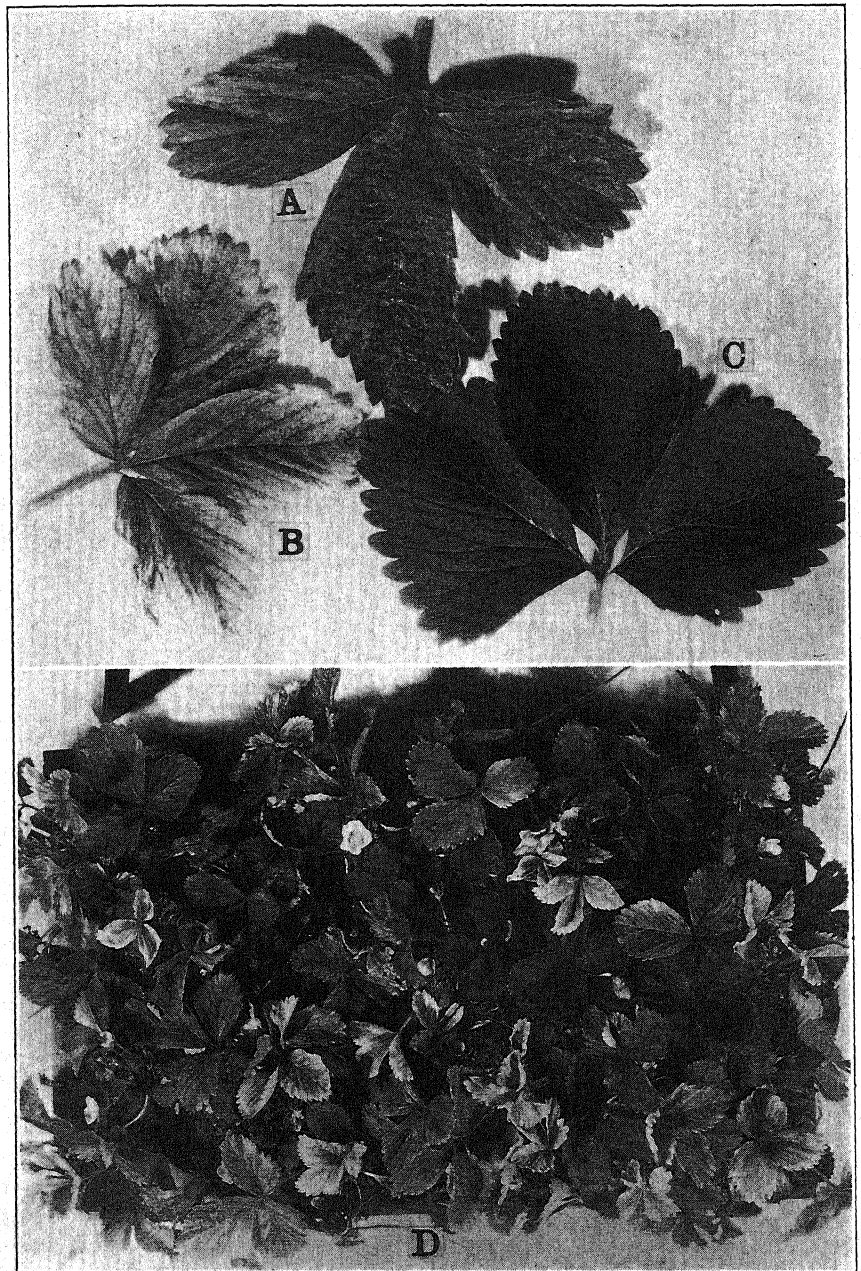


FIG. 1. Symptoms of strawberry yellows. A. Puckered and streaky-mottled leaf. B. Grayish white appearance around margin of leaf. C. Healthy leaf. D. Diseased plants of Howard 17 strawberry as they appear in the row in the field.

In Massachusetts this same disease referred to as "gold leaf" by Guba (4) is confined to the Howard 17 variety, expressing itself in plant degeneration and crop depreciation. In districts outside of New England it has been reported especially on Van Dyke, Eaton, some other new varieties, and many unnamed seedlings, none of which is grown in Massachusetts. Of particular interest is the fact that chlorosis is manifested in late April after the new beds are planted but disappears by the first week in July. This periodic appearance and disappearance of the disease is certainly not characteristic of viroses. The foliage, blossoms, and fruit show all stages of dwarfing, according to the intensity of the chlorotic condition, but no manifestation of so-called mosaic ever has been noticed in the fruit. Plants from a chlorotic source are much more difficult to establish in new plantings than normal plants, resulting in broken rows or skips (Fig. 2). There is also some mortality among chlorotic plants in the beds.



FIG. 2. Howard 17 strawberry bed in mid-June showing skips where severe chlorosis has resulted in plant mortality, and patches of chlorotic plants.

The chlorotic condition is quite general on the Howard 17 variety in eastern Massachusetts but its prevalence in strawberry beds is variable. In some beds it is general but in the majority of them its distribution is localized to one or more spots. Beds completely free of the trouble are uncommon.

In the writer's studies, seed from affected plants produced both chlorotic and heathy seedlings (Fig. 3), and the characteristic symptoms were manifested as soon as the seedlings developed their first true leaves. The tendency to dwarfing was very pronounced and ultimately the chlorotic seedlings died, their mortality being associated with almost complete absence of chlorophyll (Fig. 3). This inhibition in the development of chlorophyll is known to be characteristic of genetic chloroses in contrast to a degenera-

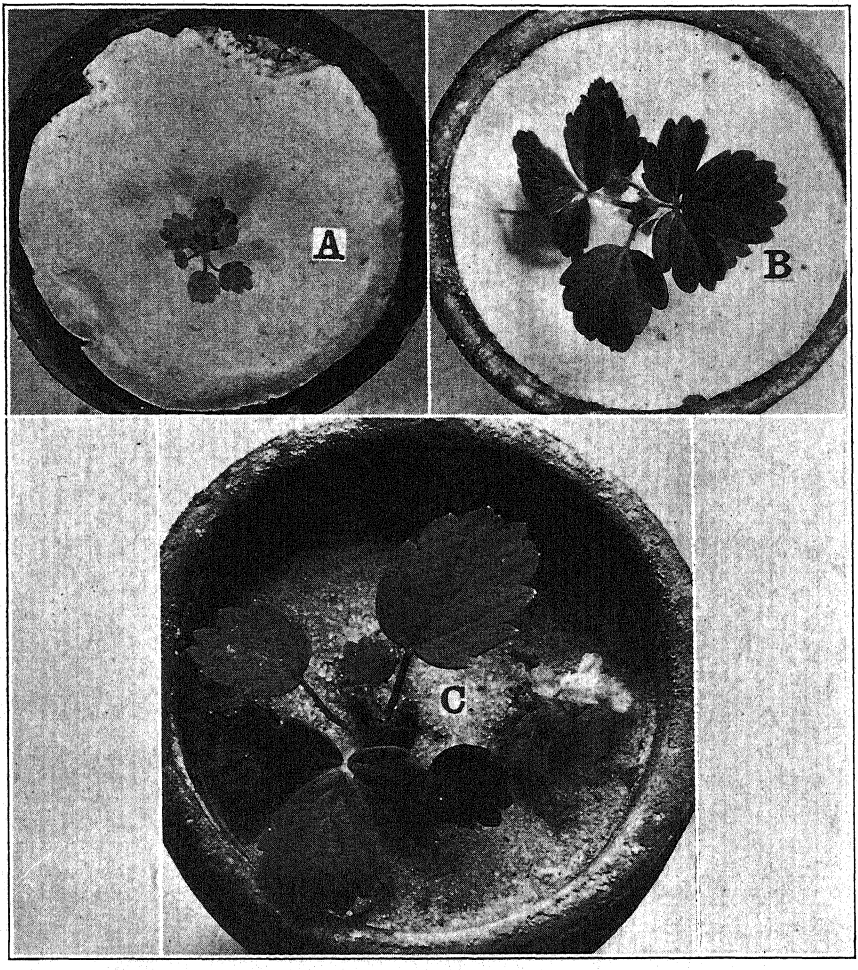


FIG. 3. Strawberry seedlings from a chlorotic parent. A. Chlorotic seedling. B. Normal seedling of same age as A. C. Normal and chlorotic seedlings of same age in same pot. The chlorotic seedling at the right is extremely dwarfed and almost entirely free of chlorophyll.

tion of chlorophyll as in viroses and is strong evidence in favor of a genetic hypothesis. Microscopic cross-sections of leaves from chlorotic and normal seedlings reveal a distinct contrast in the number of chloroplasts, (Fig. 4), and in the growth of the leaf tissues.

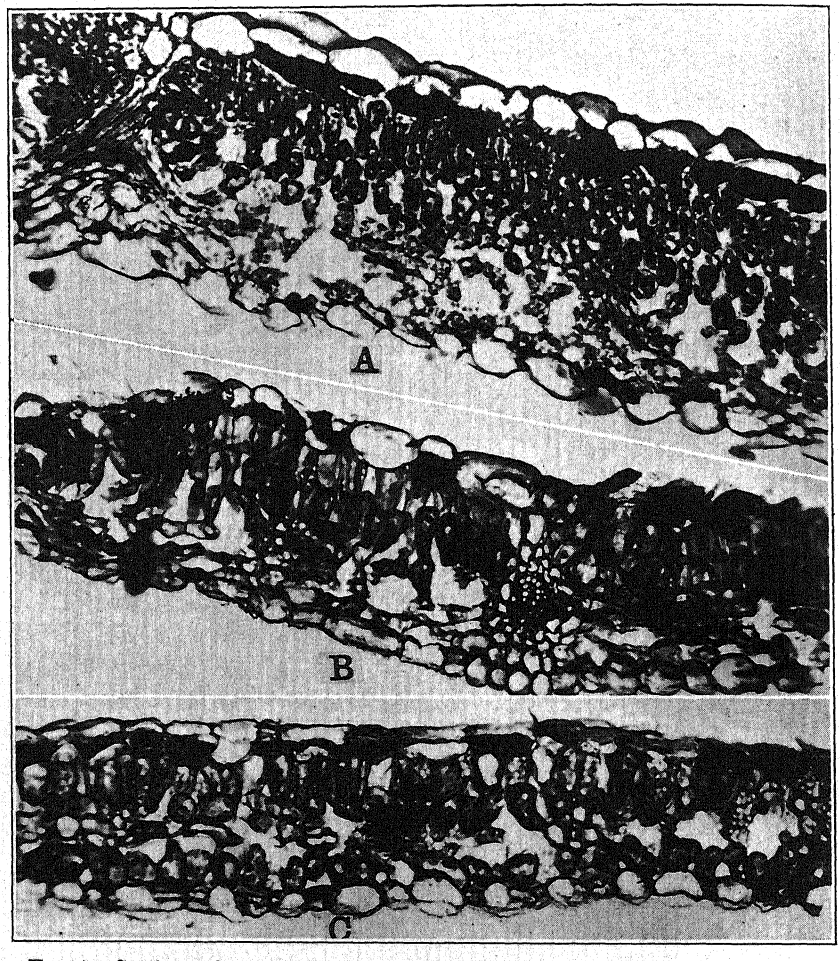


FIG. 4. Sections of normal and chlorotic leaves of strawberry seedlings. A. Normal leaf showing abundance of chloroplasts. B. Chlorotic leaf entirely free of chloroplasts. C. Chlorotic leaf showing a less complete inhibition in development of chloroplasts. × 150.

Efforts at artificial transmission of the trouble by possible insect vectors, notably aphids, white flies, tarnished plant bugs, leaf hoppers, and red spiders, and by inoculation with sap from diseased plants failed in all in-

stances.[3] Fertilizer dressings of iron sulfate, basic slag, dolomite lime, potassium permanganate, and manganese sulfate failed to effect any change in the expression of the trouble.

Certain observations in Massachusetts in the past few years would seem to throw further light upon the nature of the disease. A completely chlorotic bed was observed in the spring of 1932. This bed was planted in 1931 with seedlings that had come voluntarily from a normal green bed of Howard 17, which had been plowed under in 1930 because of rot in the berries. In previous years, when vegetative propagation was practiced, the grower had never observed the condition in his stock but when he resorted to sexual propagation it appeared suddenly. This observation is in agreement with the reports of Clark and Berkeley of instances of the sudden development of chlorosis among seedling varieties. The extent to which sexual propagation of strawberry plants is practiced is not known. Strawberry seedlings will grow where an old bed has been plowed under or where cull berries are dumped during the harvest season, and it is possible that some such plants have been used for propagating new beds. The practice of removing blossoms during the first year is by no means thorough enough to prevent the appearance of volunteer sexual plants in the rows. It is barely possible that some growers have used such plants in propagating from 2-year-old beds. If the hypothesis of a genetic condition is correct, sexual propagation would suggest an adequate explanation for the trouble.

The chlorotic condition is systemic. Where its prevalence is slight it does not become economically important unless a large number of daughter plants from chlorotic mother plants are used in the new planting. It is impossible for the grower to avoid these because the chlorotic condition does not appear until after the new beds are planted in the early spring. Further, in two efforts in 1930 and 1931 no success in the control of chlorosis attended selection and rogueing in the preparation of new beds when the vegetative stock used was originally from a moderately prevalent chlorotic source. In spite of these efforts, the disease was very prevalent in the spring of the bearing year.

In 1930 a bed of Howard 17 was set with daughter plants from a source showing a faint evidence of chlorosis. The chlorotic condition was not evident in the bed until the spring of 1931. In that season the chlorotic areas were definitely marked off. In 1932 spread appeared but it was too remote from the original chlorotic areas to have a systemic connection with plants showing chlorosis the previous season. Another bed was planted in 1931 with carefully selected chlorosis-free daughter plants from a bed in which the disease was present. During the spring of 1931 all plants suspected of

[3] Studies with insects were conducted under the direction of W. D. Whitcomb of the Department of Entomology.

showing chlorosis were removed. In the early spring of 1932 chlorotic daughter plants appeared to originate from normal green mother plants but, in the latter part of June, the chlorotic condition appeared generally in the rows.

These observations would suggest that these plants were potentially chlorotic but that the expression of the trouble was masked by the environment. It is difficult to believe that the trouble is due to an infectious virus in view of its behavior, of the negative evidence of transmission offered by Plakidas, Berkeley, and the writer, and of the fact that the condition has been developing among certain of the newer seedling varieties.

The manifestations of strawberry chlorosis are controlled or modified by the environment. Dry spring weather conditions seem to mask and modify the intensity of the trouble and to delay its appearance, while moist weather conditions favor its expression. Observations clearly indicate that potentially chlorotic plants do not always become chlorotic.

The disease is unquestionably of genetic origin and undoubtedly the result of a recessive genetic factor the expression of which is influenced by the environment. Where normal green plantings are being maintained the original heterozygous Howard 17 clon is undoubtedly being preserved by vegetative propagation exclusively. This clon should never be expected to show chlorosis so long as strictly vegetative propagation is practiced, although it would always bear the potential factor for chlorosis. Sexually propagated stock from this clon could show the trouble in varying amount, according to environmental conditions, and it is most likely that self fertilization rather than cross fertilization has contributed to the source of the trouble. According to this view, new varieties and seedlings genetically related to Howard 17 would always be liable to chlorotic outcropping.

If this hypothesis be correct, it is possible that some particular plant grower in eastern Massachusetts originally distributed sexually propagated plants of the original Howard 17 clon whence it has spread; and, since the disease is systemic, it has maintained and gradually increased itself vegetatively in these plantings. This hypothesis could account for the general prevalence of the disease in this section of Massachusetts in Howard strawberry beds. It would not be necessary to transfer chlorotic stock from locality to locality to spread the trouble, since, wherever the Howard variety is grown, some voluntary sexual reproduction with outcropping of chlorosis could occur. According to this hypothesis new varieties and seedlings containing the factor for chlorosis would always be liable to chlorotic outcropping and it would explain the distribution of the trouble among different varieties in the northeastern states and Canada, according to the survey report of Plakidas. Some particular instances of sexual propagation must

have occurred in eastern Massachusetts to account for the prevalence of the condition on the so-called Howard variety in this area.

DEPARTMENT OF BOTANY,
MASSACHUSETTS AGRICULTURAL
EXPERIMENT STATION, FIELD STATION,
WALTHAM, MASSACHUSETTS.

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FURTHER STUDIES OF LIMA-BEAN SCAB

ANNA E. JENKINS[1]

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INTRODUCTION

New data on the geographic range and symptoms of the disease known as scab of Lima bean (*Phaseolus lunatus macrocarpus* Benth.) and on the life history of its pathogen tentatively identified as *Elsinoë canavaliae* Rac.[2,3] are here reported.

GEOGRAPHIC RANGE

The geographic range of the scab of Lima bean has been found to include Central America, the Dominican Republic, and possibly Jamaica, as well as the previously reported localities, namely, Cuba,[2] Puerto Rico,[2] and Sinaloa, Mexico.[4]

That the disease is more or less widely distributed in Central America and occurs there on the Lima bean growing both wild and cultivated was ascertained by a recent examination of dried specimens of *Phaseolus lunatus macrocarpus*[5] from El Salvador, Nicaragua, Costa Rica, and Guatemala, deposited in herbaria in the United States.

The specimen from Guatemala, 1890, now constitutes the earliest known record of the disease. Figure 1, A, shows an infected leaf of the Nicaraguan specimen, and figure 1, B, an infected pod of that from El Salvador.

[1] Acknowledgment is made to C. A. Weatherby, Curator of the Gray Herbarium, Harvard University, Cambridge, Mass., and W. R. Maxon, Curator of the U. S. National Herbarium, for fragments of specimens of *Phaseolus lunatus macrocarpus*, from Central America, showing lesions of Lima-bean scab. These have been deposited in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Jenkins, A. E. Lima-bean scab caused by Elsinoë. Jour. Agr. Res. 42: 13–23. 1931. (On page 20, paragraph 2, line 4, of this paper the word "here" should be changed to "where," and in line 7, "this structure was" be inserted after "structure").

[3] The Lima-bean scab organism is described as a distinct species in a related paper by S. C. Bruner and A. E. Jenkins to be published in the Journal of Agricultural Research, vol. 46.

[4] Jenkins, A. E. Additional data on the range and prevalence of Lima-bean scab. Phytopath. 21: 559. 1931.

[5] El Salvador, San Salvador (cultivated), 1925, Salvador Calderón 2250. In Gray Herbarium, Harvard University, Cambridge, Mass.

Nicaragua, Masaya, Jan. 27, 1903, C. F. Baker 117. Field notes: "A tall vine spreading thickly over low trees." In Gray Herbarium and in U. S. National Herbarium, Washington, D. C.

Costa Rica, San José, buissons au bord du rio Maria Aguilar, Nov. 27, 1894. A. Corduz 8964. In U. S. National Herbarium.

Guatemala, Guatemala, Feb., 1890. J. D. Smith 2297. In U. S. National Herbarium.

Information in regard to the occurrence of the disease in the Dominican Republic was secured through R. Ciferri. Sending representative specimens, he wrote[6] that as early as 1925 or 1926 he had observed this disease on leaves, stems, and green pods of the Lima bean but that on the basis of a superficial examination fungi were not apparent on the specimens collected and the disease was not diagnosed until the publication of the writer's[7] first report on it. Ciferri also stated that doubtless the disease is wide-spread in the Dominican Republic, that a large percentage of beans offered for sale in the interior markets are affected, and, further, that the disease is particularly common in the wet districts of the northern and southern parts of the Republic and rare in semixerophytic zones, as at Santo Domingo.

The record of the possible occurrence of the disease in Jamaica is based on the fact that scabbed Lima beans, said to have come from that country, were recently intercepted in a passenger's baggage by a port inspector of the United States Plant Quarantine and Control Administration.

Inquiries have been made in regard to the occurrence of the disease in South America and in West Indian Islands other than those already mentioned, but thus far no record of any Elsinoë disease on Lima bean in these regions has been obtained. As yet no record of the occurrence of Lima-bean scab in the Orient is available.

The continued prevalence of the disease in Cuba is indicated by the fact that typically spotted pods have been frequently intercepted during the shipping season, November 1, 1931, to March 31, 1932, by inspectors of the Plant Quarantine and Control Administration of the United States Department of Agriculture, at the Port of New York. The disease has also been common on Puerto Rican Lima beans received at the same port during the period named.

In the summer of 1931 intensive search for scab was made in commercial Lima-bean fields in Georgia, South Carolina, North Carolina, Virginia, New Jersey, and New York, by J. H. Miller and B. S. Crandall, both at that time of the Plant Disease Survey, and by W. D. Moore, of the Division of Horticultural Crops and Diseases, Bureau of Plant Industry. The writer examined specimens of leaves, stems, and fruits received from these investigators and other American-grown Lima beans secured in markets at Washington, D. C., and elsewhere in the East. No trace of the disease was found on these American-grown beans. W. A. McCubbin, of the Plant Quarantine and Control Administration, surveyed Lima-bean-growing regions in California in 1931 but did not find the disease there.

[6] Letter dated Nov. 21, 1931.

[7] Jenkins, A. E. *Loc. cit.* (Footnote 2).

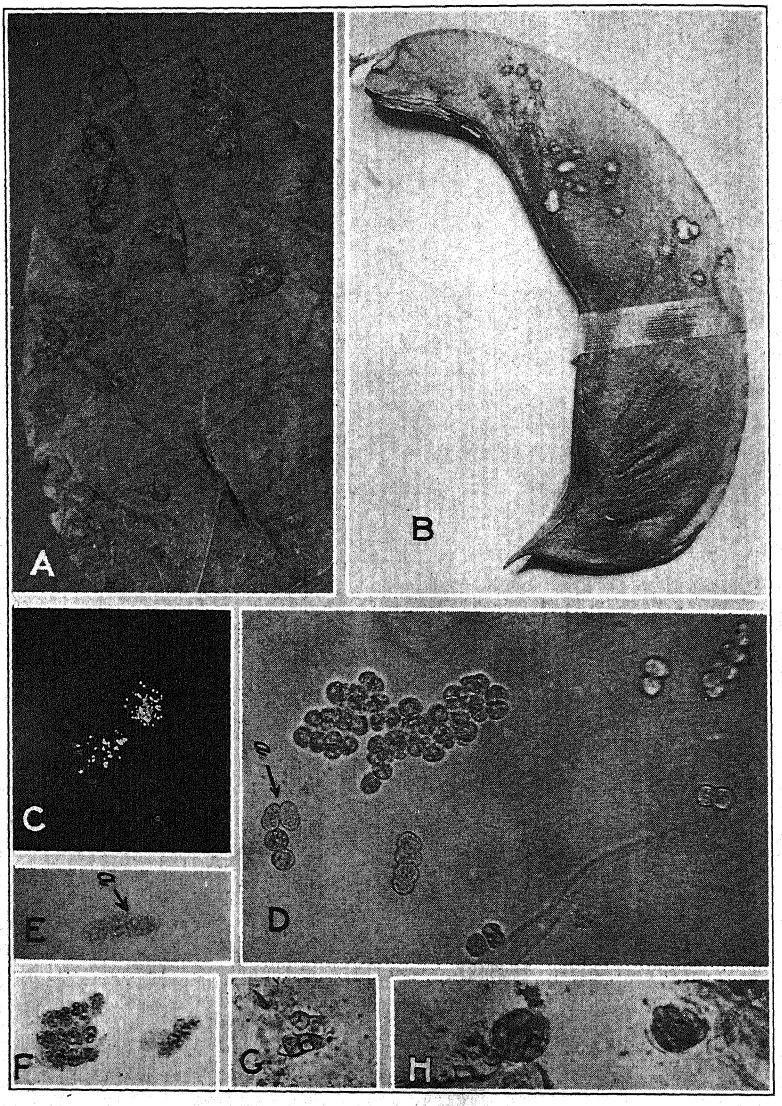


FIG. 1. A and B. Scab lesions on dried specimens of Lima bean (A) from Nicaragua, 1903 (×about 2) and (B), El Salvador, 1925 (×about 1). C–F. Cultures of the Elsinoë causing the disease from ascospores discharged from pod lesions to corn-meal-agar surface. ×380. D, *a*. Swollen sprout conidia. E, *a*. Refringent granules. G and H. Reddish-brown ascospores from pod lesions, those in H within ascus. ×380. For further explanation, see text.

SYMPTOMS

During the shipping season of November 1, 1930, to March 31, 1931, further studies showed that, while scab lesions may be noticeably swollen

or thickened, they are not the cause of the projections on pod margins, although they may sometimes be found on these regions as well as on other parts of the pod. The earlier material had seemed to indicate that such development resulted from attack by the fungus, although, even then, jaggedness of the pod margins was found independent of the lesions. The same phenomenon also has been noticed by S. C. Bruner in connection with his study of the disease.

LIFE HISTORY

In order to obtain some data on the longevity of the Elsinoë pathogen of Lima-bean scab, tissue cultures were made early in April, 1930, from fresh, diseased Lima beans from Cuba, received through the Plant Quarantine and Control Administration. Late in August, 1930, additional tissue cultures were made from the dry pods, some of which had been kept at room temperature and others at 17° C. The fungus was isolated only from the fresh pods. This result suggests that the pathogen was not viable on the dry pods, which were nearly 5 months old.

While the pods just referred to were fresh, additional cultures were made from them, mainly to determine whether ascospores would become distinctly colored with age. Ascospores for observation were obtained by placing pod lesions so that the ascospores from them could be discharged upward (less than half an inch) to water on a glass cover slip and to corn-meal-agar medium on glass slides.

After the spores had been discharged on these media, the cultures were placed in Petri dishes lined with filter paper and were held at a temperature of about 8° C. When placed at this temperature the ascospores, then not noticeably swollen, had in some cases begun to sprout conidia from their surfaces. Twenty-four hours later in the water culture and 5 days later in the agar culture both ascospores and conidia had become considerably swollen. In the agar culture, conidia were sometimes seen lying in a row at the side of an ascospore, evidently in the position in which they were produced. Figure 1, D, shows the appearance of ascospores in the agar culture when 8 days old, and figure 1, E and F, their appearance when 30 days old. In the older cultures the ascospore contents were glistening and coarser than in the younger cultures, and in some cases they appeared greenish. (Fig. 1, F). In no instance, however, did they show any deeper color than pale yellow, in either the water or the agar cultures. Petri-dish cultures, corresponding to those just described but grown at room temperature, served only to show that the inoculum was purely of the Elsinoë, for growth took place too rapidly for continued observation of the ascospores. The colonies when 7 days old were readily visible (Fig. 1, C) and were covered with conidia.

In another experiment fresh pod lesions were held for a few days in a moist chamber, and ascospores from them were then examined for coloration. These ascospores were reddish brown (Fig. 1, G and H), the coloration possibly having been derived from the pigment in the lesions. Similar observations have been reported for Myriangium[8] and Uleomyces.[9] Further study of the Alternaria commonly associated with the Elsinoë on its natural substrate shows that its conidia are sometimes easily confused with the colored muriform ascospores of the Elsinoë. A colored muriform spore, once thought[10] to be an ascospore of the Elsinoë, is now believed to be an Alternaria conidium and not one of the colored muriform Elsinoë ascospores of about the same size present on the same slide.

In order to obtain some data on the temperature relations of the Elsinoë, test-tube slant cultures of the fungus on potato-dextrose-agar medium were grown at constant temperatures of 0°, 5°, 10°, 15°, 20°, and 25° C. The inoculum for each culture consisted of a small amount of cultural growth about 0.5 mm. in diameter. After 3 weeks, the 2 sets of cultures, each made from a different isolation, showed no growth at 0°, slight growth at 5°, and increasing amounts of growth up to 25° C.

SUMMARY

Costa Rica, Nicaragua, Guatemala, and El Salvador, in Central America, the Dominican Republic, and possibly Jamaica are added to the known range of Lima-bean scab, previously reported from Cuba, Puerto Rico, and Sinaloa, Mexico.

Although lesions on pods may be noticeably swollen or thickened, they are not the cause of projections on pod margins, as previously reported.

Failure to isolate the pathogen of the disease from diseased dry pods held for nearly 5 months, some at room temperature and others at 17° C., suggests that the fungus was no longer viable on this material. Ascospores removed from pod lesions to culture media (water and corn-meal agar) and held at about 8° C. to check growth remained hyaline or pale even when a month old, whereas ascospores in fresh pod lesions kept moist for a few days were reddish brown, possibly deriving their color from pigment in the lesions. Cultures grown for 3 weeks at the constant temperatures of 0°, 5°, 10°, 15°, 20°, and 25° produced no growth at 0°, slight growth at 5°, and increasing amounts of growth up to 25° C.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

[8] Petch, T. Studies in entomogenous fungi. V. Myriangium. Brit. Mycol. Soc. Trans. 10: 45–80. 1924. (Pp. 62–63).

[9] Jenkins, A. E. Scab of Canavalia caused by *Elsinoë canavaliae*. Jour. Agr. Res. 42: 1–12. 1931. (P. 7).

[10] Jenkins, A. E. *Loc. cit.*, pl. 5, C. (Footnote 2).

NEW LOCATIONS FOR PHYTOPHTHORA CITROPHTHORA AND P. HIBERNALIS ON CITRUS[1]

HOWARD S. FAWCETT[2]

(Accepted for publication August 31, 1932)

Phytophthora citrophthora (Sm. and Sm.) Leonian has been found recently in Florida and Louisiana, and *P. hibernalis* Carne, in California.

During a trip to Florida in January, 1932, occasional examples of a decay of oranges and grapefruit resembling citrus brown rot were noticed, but only on fruit that was lying on wet soil under trees at the edge of swamps or drainage ditches. Isolation culture tests gave pure cultures of *Phytophthora citrophthora* in three widely separated districts in Florida and cultures of *P. parasitica* Dastur in one other district.

Phytophthora citrophthora was obtained at Wildwood, Vero Beach, and Palmetto, Florida, and at Buras, Louisiana. Near Wildwood the oranges from which the fungus was obtained had been lying on moist soil in a large hammock grove where the orange trees were growing among the native oaks and palmettos and on the edge of swampy land. Near Vero Beach and Palmetto, grapefruits from which the fungus was isolated had been lying on very moist soil at the margins of drainage ditches.

In Louisiana, where heavy rains had been recent, the same fungus was isolated many times from oranges showing brown rot. Some of the fruits were obtained from the orchard and others from the packing houses in the vicinity of Buras in the delta region below New Orleans.

The cultures of *Phytophthora parasitica* were obtained from orange fruits with a decay resembling brown rot, on wet soil near Courtney, Merritt's Island, Florida.

The best method of obtaining a pure culture from these fruits was to take out a seed aseptically by means of sterilized scalpels, tweezers, etc., and to drop this seed on a slant tube of glucose-potato or corn-meal agar. If the fruit was seedless a piece of the albedo, core, or inner membrane was used.

These isolations from Florida and Louisiana were compared by growing them in parallel cultures with known strains of *Phytophthora citrophthora* and *P. parasitica* on corn-meal and glucose-potato agar. They were then grown in dilute prune extract, a bit of the mycelium transferred to tap water in shallow dishes and in from 24 to 48 hours (at 18° to 20° C.) all of them formed abundant sporangia. All of them appeared to be typical *P.*

[1] Paper No. 273, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California. Published with the approval of the Director of the Station.

[2] Professor of Plant Pathology.

citrophthora except the isolation from Courtney, Merritt's Island, Florida. Lemon and orange fruits were then placed in the water of the shallow dishes containing these sporangia and typical brown rot was produced in from 3 to 6 days from all isolations. The brown rot produced by *P. parasitica* usually tended to be a little lighter in color than that due to *P. citrophthora* but was very similar otherwise. These isolations were sent to Dr. S. F. Ashby of the Imperial Mycological Institute, who confirmed the author's identification of these strains. This appears to be the first authentic record of *P. citrophthora* in Florida.

Orange fruits having a decay resembling brown rot had been noted in Florida before, once by H. J. Ramsey and once by H. R. Fulton. The latter isolated a Phytophthora from two grapefruits collected at Orlando, Florida, and concluded that it was *P. parasitica.*[3]

The occurrence of *Phytophthora citrophthora* has now been reported in many citrus-growing countries of the world. The fungus was first described and reported by Smith and Smith[4] in California in 1906. It has since been reported in Arizona by Fawcett, Brazil by Averna-Sacca, Chile by Opazo, Union of South Africa by Doidge, Australia by Carne and others, Spain by Fawcett, Italy by Petri and by Fawcett, Egypt by Sirag-el-Din, Palestine by Reichert, Hawaii by Pope, Japan by Sawada, and the present report adds Florida and Louisiana.

In February, 1932, following the snows and rains of January, attention was called to the prevalence of brown rot on orange trees in the Cucamonga district of California. Not only were the fruit and leaves attacked in the usual manner, *i.e.,* up to a height of 3 feet from the ground, but frequently places were noted where the attack was much higher, occasionally nearly to the top of the trees. It was suspected that some other Phytophthora was present, and not the usual brown rot fungus. Although the appearance of the decay resembled that produced by *P. citrophthora* and *P. parasitica* (the organisms most commonly associated with citrus brown rot in California), isolation culture tests showed that another Phytophthora was present. This was sent to S. F. Ashby, who identified it as *P. hibernalis* Carne.[5]

This appears to be the first definite record of this fungus on citrus in America. This species was first described by Carne in western Australia. It has been found also in other parts of Australia. It was determined by

[3] Fawcett, H. S., and H. Atherton Lee. Citrus diseases and their control. P. 373. McGraw Hill, New York, 1926.

[4] Smith, R. E. and E. H. A new fungus of economic importance. Bot. Gaz. 42: 215–221. 1906.

[5] The author is indebted to Dr. S. F. Ashby of the Imperial Institute of Mycology, Kew, Surrey, England, for identification of this fungus and for confirmation of the identity of the isolations of *Phytophthora citrophthora* from Florida and Louisiana.

Carne to be the same as that reported as *Phytophthora* sp. by Monz da Maia in Portugal. Later, Bensaude reported the same fungus on both leaves and fruit in Portugal. This species is remarkable in having a very low optimum temperature for growth of about 12° C. as recorded by Carne. It is significant in this connection that the occurrence in California followed closely a period of cool weather with an unusual amount of snow and with frequent rains. Cultures of this fungus have been found to die in a short period at a temperature of 25° C. This heavy attack of the leaves near the ground on orange trees has been noted by the author in previous years and he believes now that *P. hibernalis* may have been in California for a number of years without having been recognized. It is also remembered that a similar fungus was isolated at Oroville, California, in a very cold spring, about 1922, and that the fungus soon died at room temperature of summer (about 25° C. at Riverside) before it could be studied. It is now believed that this isolation was *P. hibernalis*. This fungus is so sensitive to the ordinary temperatures of late spring and summer in California that it could presumably not exist except at some depth in the soil or by means of the resistant oospores during those seasons of the year.

Adding the above information to that previously reported, there are now known to be on citrus fruits in California at least three species of Phytophthora, all capable of producing citrus brown rot. These are *Phytophthora citrophthora, P. parasitica* (*P. terrestris*), and *P. hibernalis*.[6] These three have also been reported in the Mediterranean region. Two of these, *P. citrophthora* and *P. parasitica,* appear to have a wide distribution, having been reported already in most of the other citrus-growing districts of the world, including Florida. Other species of Phytophthora that have been reported so far on citrus are *P. citricola* in Japan, *P. palmivora* (*P. faberi*) in the Philippines and West Indies, and *P. arecae* in India.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

[6] A Phytophthora isolated from roots of citrus in Tulare County, Calif., has been identified also by Dr. Ashby as probably *P. megasperma* Drechsler.

PHYTOPATHOLOGICAL NOTES

An Apparent Case of Transmission of Cranberry False Blossom through a Natural Graft.—All attempts to transmit false blossom of the cultivated cranberry, *Vaccinium macrocarpon* Ait., by methods other than insect transmission with the leaf hopper, *Ophiola striatula* (Sleesman) Fall., as the vector have failed. The usual methods for artificially transmitting virus diseases, such as pricking or scratching with a needle, rubbing or other method of leaf mutilation, and even injecting virus by means of a hypodermic needle have produced no results. Some other virus diseases are similar to false blossom of the cranberry in this respect. Thus, Kunkel[1] mentions various methods used to transmit aster yellows artificially and says: "All attempts to transmit aster yellows mechanically, except by budding, have failed." Peach yellows also cannot be transmitted artificially by any method of rubbing, puncturing, or injection, although it is readily transmitted by budding or grafting. The only known attempt to transmit false blossom by grafting is that reported by Dobroscky,[2] who states that: "Grafting experiments were attempted several times. Cranberry stems are very woody, wiry and thin. Inarching grafts were made but the plants did not grow together. No infection resulted even though the stems were in external contact for long periods of time. If the grafts had been successful infection might have resulted."

The discovery of a natural graft involving apparent transmission of false blossom is, therefore, of considerable interest. The natural graft was found among some young vines of the Wales Henry variety that had been grown on the State bog at East Wareham, Massachusetts, from stem cuttings planted in May, 1931. In preparation for planting, the old vines were removed from several square yards of the bog and a layer of fresh sand, to a depth of about 2 in., was placed over the peat substratum. The cuttings were made from selected apparently healthy vines, although it was found later in the summer that a number of the cuttings had been made from infected vines. The cuttings, 6 to 8 in. long, were inserted singly in holes to a depth of about 2 in. into the peat below the sand covering. In plantings as ordinarily made 2 or more cuttings are inserted in a hole. In one instance, here described, 2 cuttings happened to be inserted together so that the stems were crossed, forming an X. One of the cuttings (Fig. 1, A) was apparently from a vine infected with false blossom, as the plant from

[1] Kunkel, L. O. Studies on aster yellows. Amer. Jour. Bot. 13: 646–705. 1926. See pp. 679, 681.

[2] Dobroscky, Irene D. Studies on cranberry false blossom disease and its insect vector. Contr. Boyce Thompson Inst. Plant Res. 3: 59–83. 1931. See p. 64.

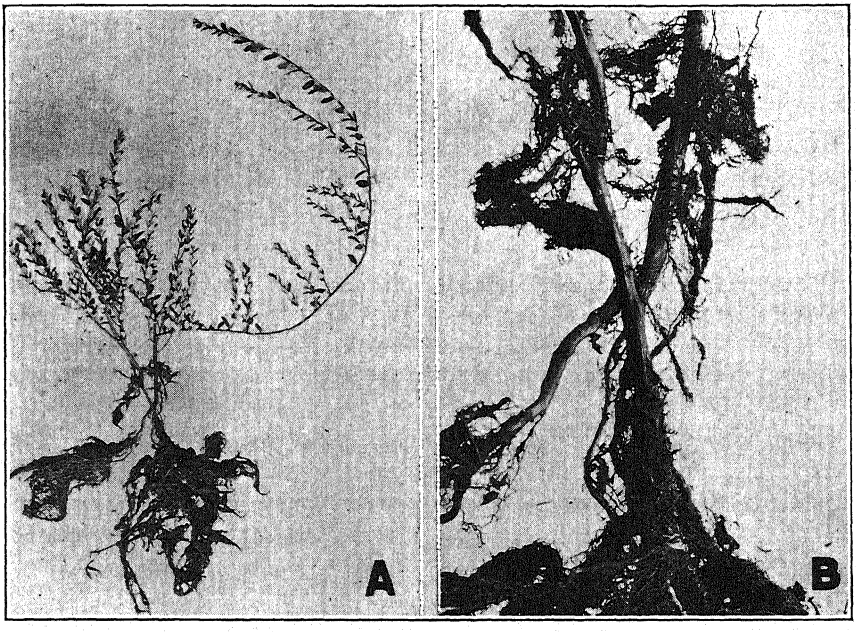


FIG. 1. A. Two young cranberry plants united by a natural graft. False blossom has apparently been transmitted to the plant at the right from the originally diseased plant at the left through the graft. About × ½. B. Closer view of the naturally grafted stems. × 2.

this cutting showed throughout marked symptoms of the disease and produced 1 flower that was very characteristic of the disease. The other cutting (Fig. 1, B) seems to have been healthy when it was set out. This plant showed very definite infection on several uprights from a runner near the base of the plant. Branches above this runner, the distal portion of the runner itself, and 1 upright from it, most remote from the point of origin of the runner, showed no evidence of infection. From this it seems probable that the second plant was infected sometime during the season of 1931, as in false blossom of the cranberry evidence of infection generally does not appear until the year following that in which infection occurs. The relative position of diseased and healthy portions of the second plant also indicates that the infection came from below, *i.e.*, from the direction of the line of union in the graft. Healthy and diseased cuttings in the same hill have been noticed at various times by different observers[3] and often grow for long periods of time without the disease spreading from one to the other. The occurrence of a natural graft in any such case has, heretofore, been unknown.

[3] Personal communications from Neil E. Stevens and from H. F. Bain.

There is, of course, a possibility that the cutting that developed symptoms of false blossom in 1932 may have been made from an infected vine, as was true of some other cuttings in the small area of new planting of the Wales Henry variety. This variety is very susceptible to false blossom, and the vines from which the stems were selected for propagation were badly infected with the disease. For this reason it was very difficult to select vines for propagation that could be known certainly to be free from false blossom. However, the fact that none of the vines grown from cuttings developed symptoms of false blossom, except those in which the disease appeared in 1931, makes this possibility negligible.—H. F. BERGMAN and W. E. TRURAN, Cranberry Disease Field Laboratory, U. S. Department of Agriculture, East Wareham, Massachusetts.

Two Hosts of the Pokkah-bong Disease other than Sugarcane.—Early in the summer of 1929 a disease, apparently very similar to pokkah bong, was observed on several young corn plants. A microscopic examination of some of the affected tissues revealed the presence of numerous Fusarium spores that were very similar in size and shape to the spores of the Fusarium causing pokkah bong on sugarcane.

A short time after the affected corn plants were observed a similar affection was noted on young sorghum plants growing close by, and these also, when examined under the microscope, showed the presence of Fusarium spores. The symptoms on the corn and sorghum plants were very similar to those on sugarcane caused by pokkah bong and strongly suggested that these plants might be additional hosts of the pokkah-bong fungus.

On neither the corn nor the sorghum were the chlorotic spots quite so pronounced as on sugarcane. The leaf bases on the corn were more of a yellow chlorosis, but the red and black markings were almost identical with those observed on cane. In some cases a top rot had developed, which had the same obnoxious odor as the top rot on sugarcane. Many cases were observed where the corn tops were distorted and curved and nearly always, on these plants, the lesions or cavities could be found on the surface of the stalks. (Fig. 1, A.) No infection has yet been found inside the corn stalks.

Infection on the sorghum plants, while usually not so severe, is very similar to that observed on sugarcane. The leaf bases show the chlorotic spots and red markings very plainly, and, where the red markings dry and fall out, the leaves have the same ragged appearance as the similarly affected sugarcane leaves. (Fig. 1, B.) The red markings on the sorghum leaves are much more pronounced than on sugarcane, but this is to be expected, as most all sorghum leaf diseases are accompanied by a distinct reddening of the immediate or surrounding tissues. No infection has been observed inside the sorghum stalks, but, on the more susceptible varieties

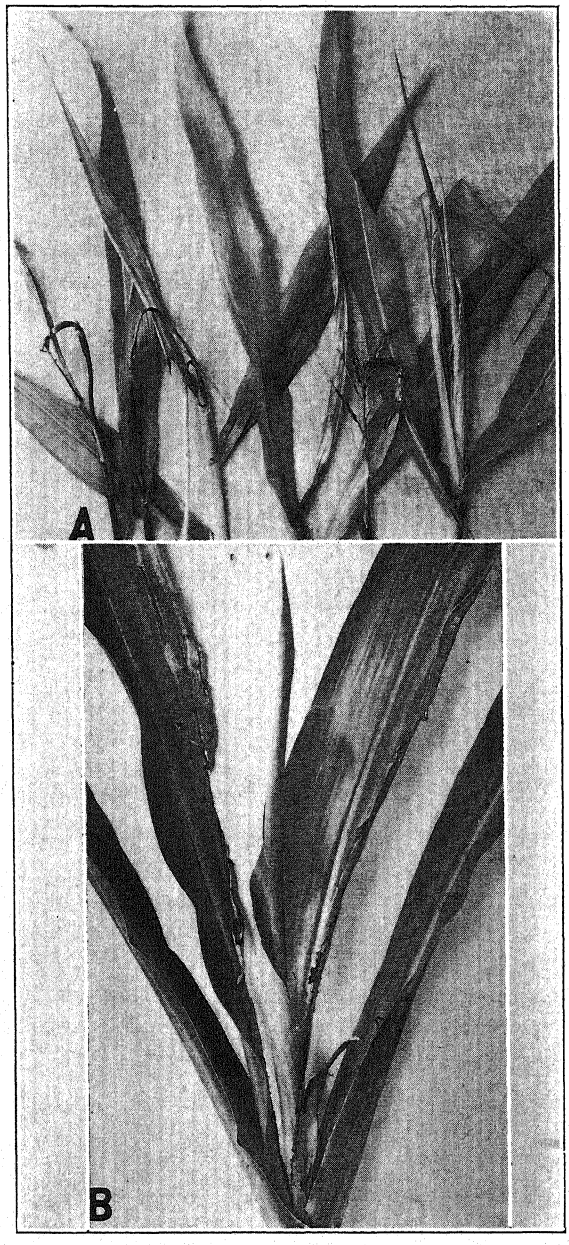


FIG. 1. Pokkah bong. A. Produced by inoculation of young corn plants. Note distortion and rot on some plants. B. On sorghum. The ragged appearance is caused by the affected tissues drying and falling out.

and under favorable conditions, the fungus might penetrate to the inner tissues of the stalks.

Numerous isolations have been made from infected plants of both the corn and sorghum, and in nearly every case a Fusarium fungus was obtained. This was isolated in pure culture and inoculated back onto several corn, sorghum, and sugarcane plants. The symptoms produced on the corn and sorghum from these inoculations were identical with those from natural infection, while those on the sugarcane were typical of pokkah-bong infection. Pure cultures were then obtained from sugarcane plants affected with pokkah bong and inoculated onto several corn, sorghum, and sugarcane plants. These inoculations produced almost exactly the same symptoms as were obtained from the inoculations with the Fusarium spores isolated from the corn and sorghum.

These experiments were repeated several times with similar results in each case. The fungus isolated from sugarcane plants affected with pokkah bong produced typical pokkah-bong symptoms when inoculated onto corn and sorghum, while the fungus isolated from the affected corn and sorghum plants produced similar symptoms when inoculated onto sugarcane. It is very evident from the results of these experiments that the pokkah-bong disease of sugarcane is capable of attacking both corn and sorghum and that these plants are additional hosts of the pokkah-bong organism.—C. N. PRIODE, Cuba Sugar Club Experimental Station, Central Baraguá, Cuba.

Cuban Streak.—In the fall of 1931 a peculiar type of striping was observed on the leaves of a single stalk in a stool of POJ 2725 sugarcane growing in the experimental plots of the Tropical Plant Research Foundation at Central Jatibonico. The stripes were unlike those manifested by any other cane disease I have had under observation in Cuba and appear to be those of a disease new to the island. For convenience in describing, it has been named "Cuban streak." At the time the disease was first observed a careful inspection was made of all the surrounding plants in the field, but no other cases of infection were found. A few weeks later a similar striping was observed on a few plants of the CAC 323 variety growing close by. The field has been inspected several times since, but no new infections have been discovered.

The stripe-affected plants grew in a field that was surrounded by cane showing a high percentage of mosaic infection. The symptoms of Cuban streak, however, were very different from those of the mosaic, in that they occurred as distinct, chlorotic stripes with no indication of mottling, such as is characteristic of sugar-cane mosaic. It was first thought that the malady might be the same as the corn stripe disease, but a comparison of diseased leaves from corn and cane plants showed the symptoms to be very dissimilar.

The dissimilarity of symptoms, however, might be due to a difference in the host manifestation when the disease passes from corn to cane, and later experiments may prove the two maladies to be identical. This is not expected, however, for, as yet, all attempts to transmit corn stripe from corn to cane have given negative results. Cane leaves affected with Cuban streak have also been compared with fixed specimens of the African Streak disease preserved in jars in the laboratory of this station. The stripes of these two affections appear to be quite unlike, and it is believed that they result from the attacks of different causal agents. The insect known to transmit the African Streak disease is not known to occur in Cuba at the present time.

The attacks of the Cuban streak so far observed have been very mild, and the infection has not appeared to spread readily to the surrounding cane. Comparatively little is known about the disease, however, and it must be kept under close observation until its economic importance has been determined.

Cuban streak occurs in the form of interrupted stripes on the leaves. These appear to follow the leaf veins and in most cases extend the full length of the leaf. (Fig. 1.) The color of the stripes is a chlorotic white,

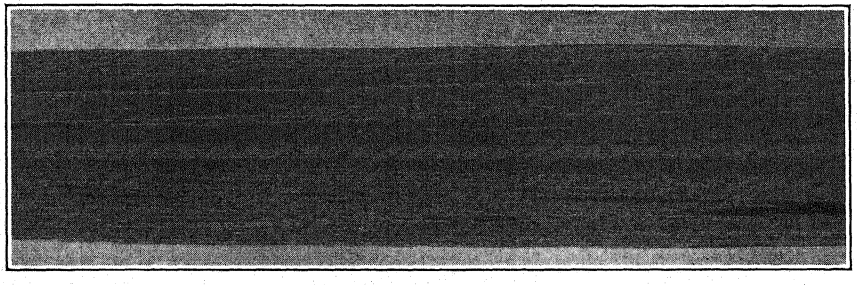


FIG 1. Cuban Streak on POJ 2725 cane. The dark stripes in the picture showed red on the cane leaf.

which shows very plainly against the normal green of the healthy tissues. In some cases some of the stripes change to a brick red, as shown by the dark stripes in figure 1. Nearly all of the leaves on the infected plants usually show from a few to several stripes. The chlorotic white of the stripes shows more distinctly on the dark green of the older leaves than on the lighter green of the younger leaf blades.

The stalks of the infected plants observed appeared to be considerably smaller than the healthy stalks of the same stools, but in most cases they were of about equal height.

The cause of this peculiar striping is not yet known. The appearance of the symptoms, however, strongly suggests the causal agent to be of a virus

nature. This idea is supported by the fact that when the original infected stalk was planted every bud that germinated produced young plants that showed the typical striping on their leaves. These young plants were thickly covered with the stripes until they had grown to a height of about 4 feet. The younger leaves at that time began to show fewer stripes as they came through until the symptoms finally disappeared altogether on the upper leaves of most of the plants. Several insects have been used in preliminary inoculation experiments, but no infection has yet been obtained from them. Since the disease was discovered only a short time ago, it has not been possible to carry out any extensive experiments to determine its cause. Further investigations are necessary before any definite information can be given concerning the nature of the disease or concerning its economic importance.—C. N. PRIODE, Cuba Sugar Club Experimental Station, Central Baraguá, Cuba.

Fusion of Large-cell Hyphae of the Cotton-root-rot Fungus.—In studying the morphology of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, during the past season, the writers have observed in artificial cultures numerous cases of specialized fusion of cells of the large-cell hyphae. This development was first observed in cultures of the fungus isolated from infected cotton plants obtained from fields at the U. S. Cotton Breeding Field Station, Greenville, Texas, during the season of 1931. All other isolations of the fungus made from infected cotton plants collected from widely separated localities in Texas have also shown different stages of fusion of the large-cell hyphae. This form of growth occurs to a greater extent in fresh isolations of the fungus, but seems to be entirely absent in attenuated cultures that do not form strands.

It was noted that individual cells of filaments in parallel positions often send out short projections that meet and fuse. In some cases the points of fusion were between the septa, while in others, they were opposite or near the septa; the connecting projections resembling in many respects the conjugations of certain filamentous algae. A simultaneous triple fusion of an individual cell with 2 parallel cells has been noted, after 24 hours incubation in a hanging-drop culture. (Fig. 1.) Other cases have been observed where the cells or filaments grew for several millimeters before encountering and making contact fusions with similar cells of other filaments. Usually at the time of fusion of these hyphae, the protoplasmic contents are multinucleate and appear as hyaline, granular, oval bodies, which refract light readily and give the cells a vacuolate appearance.

Although no actual nuclear fusion of the cells has yet been noted, it is possible that cytological studies may ultimately reveal such phenomena, but there is yet no indication of a reproductive function. The fusion processes appear to represent early stages in the development of the

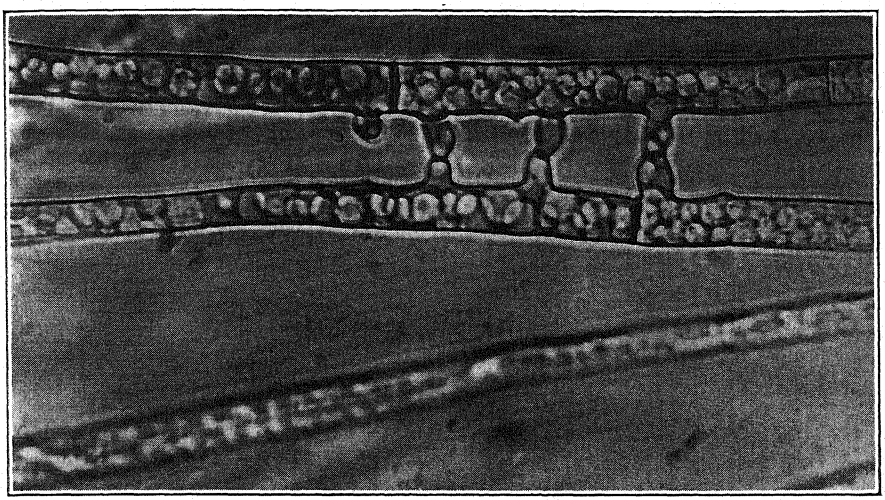


FIG. 1. Photomicrograph of large-cell hyphae of *Phymatotrichum omnivorum* showing simultaneous triple fusion of an individual cell with two parallel cells. ×570.

strand hyphae of the fungus, preceding the development of sclerotia.—D. C. NEAL, R. E. WESTER, and K. C. GUNN, Bureau of Plant Industry, U. S. Department of Agriculture, Greenville, Texas.

PHYTOPATHOLOGY

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PATHOLOGIC CHANGES IN THE ANATOMY OF LEAVES OF THE SUGAR BEET, BETA VULGARIS L. AFFECTED BY CURLY TOP[1]

KATHERINE ESAU[2]

(Accepted for publication, Oct. 1, 1932)

INTRODUCTION

Curly top of the sugar beet, *Beta vulgaris* L., induces profound anatomical changes in the affected plants, as do many other virus diseases.

The geographical distribution of curly top, its host range, and its general effects on the host plants are well known. Numerous studies have been made on the life history of the insect vector of this disease, the beet leaf hopper, *Eutettix tenellus* (Baker), and on the biological relation between that carrier and the curly-top virus. The properties of the latter have been in part determined.

The anatomy and physiology of plants affected by curly top have received, however, small attention; and there is scanty information on the processes involved in producing the histologic abnormalities.

This is a study of some important anatomical changes induced in leaves of the sugar beet by the disease known as curly top. Chief attention was given to changes in tissues in and near the degenerating phloem, after necrosis. Such a study promised to yield a general understanding of the processes by which the visible symptoms are produced. The statements of other workers concerning phloem necrosis have been checked, and several points on the normal anatomy have been cleared. A suggestion has been made that degeneration of the phloem is probably the primary effect of the virus on the plant and that certain other pathologic alterations have a causal relationship to the disturbance in the phloem. An effort has been made to

[1] Revision of the thesis submitted in December, 1931, to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] Grateful acknowledgments are made to Dr. W. W. Robbins, under whom this study was made, for his helpful guidance and criticism; to Dr. A. S. Crafts for his valuable suggestions and interest in the work; to Dr. Eubanks Carsner and to Dr. M. W. Gardner for their helpful criticism of the manuscript; to Mr. W. C. Matthews for making the photomicrographs.

correlate the knowledge gained from this study with the conception of some workers on the localization of certain viruses in plant tissues.

ANATOMY OF PLANTS AFFECTED BY VIRUS DISEASES

The morbid anatomy and physiology of virus-diseased plants in general have been somewhat neglected, as compared with other phases of virus studies. Few investigations have aimed at a morphological and physiological interpretation of the changes involved, but the pathological anatomy has often been studied incidentally in the search for a causal factor of virus diseases. In view of Quanjer's (45) recent review of these investigations, a very brief discussion of literature on the anatomy of virus-diseased plants will be given here. It shows the general similarity between the pathologic effects of curly top and of other virus diseases.

The three fundamental types of anatomic changes occurring in virus-diseased plants are (1) degeneration of certain cells or groups of cells, leading to a necrosis, (2) hyperplasia preceded by hypertrophy, and (3) hypoplasia. Necrosis is frequently confined to the phloem, as in certain potato virus diseases (30, 45, 50); but it may also occur in other tissues (35, 36, 44, 45). It may be not only internal but external, as in certain tomato (67, 68), and tobacco (34) diseases. Hypertrophy and hyperplasia, which have been described less frequently, may lead to the formation of overgrowths (29, 41) and may be closely associated with necroses (29). The green areas in leaves affected by mosaics are considered hyperplastic by certain workers (20, 21, 46), whereas the chlorotic areas have been found to be hypoplastic (9, 16, 17, 20, 21, 31, 46). The stunting and dwarfing of organs or of whole plants, effects very common to virus diseases, also are hypoplasias, in Küster's (38) terminology. The three principal types of abnormalities are usually combined, a fact which shows that the pathologic effect is not confined to one type of tissue but involves the whole plant.

EFFECT OF CURLY TOP ON THE SUGAR BEET

The following decsription of curly-top symptoms in the beet is given according to Severin (55). The first constant symptom on leaves is the clearing of tissues along the small veins, which Severin designated as "transparent venation." It generally occurs on the youngest leaves and may sometimes be confined to a portion of a leaf. It may appear on the youngest leaf of a very young plant within 2 days after inoculation and may sometimes be the only symptom visible on a leaf. Occasionally, but not always, blister-like elevations may give early indication of infection. As the disease develops, the lower surface of the leaf becomes roughened, and the veins are distorted. At first, small elevations may be observed

along the veins; these later assume the shape of nipple-like papillae and knot-like swellings resembling galls, termed by Severin "wart-like protuberances." At this stage the leaves are dark green, thick, crisp, and brittle. Frequently a clear, viscid liquid exudes from the petioles or the veins on the dorsal surface of the leaf. Inward rolling of diseased leaves usually accompanies other symptoms but occurs also in some other diseases, and is, therefore, not a sure indication of curly top. The roots of severely diseased plants are stunted and are stimulated to increased production of laterals.

Carsner and Stahl (15) state that "the disease is characterized by a necrosis of the phloem throughout the vascular system, and in the advanced stages of the disease this necrosis shows in the roots as dark concentric rings." They found that the clear, viscous exudate from the diseased petioles contains "a complex sugar, probably saccharose." In two instances traces of reducing sugars were detected. The dry weight of the diseased tops was found to exceed that of the tops of healthy plants, a fact attributed to carbohydrates, probably sugars, accumulating in the diseased leaves. The escape of the exudate was explained by "high pressure developed by the abnormal accumulation of the sugars." Leaves, mature at the time of infection, remained free from symptoms but contained the curly-top virus, which was also present in the roots.

Mumford (43) reported in diseased leaves an increase in sugars, in the concentration of total solids, and in nonelectrolytes.

Smith and Boncquet (59) described lesions in the phloem of affected plants; these were found in all parts that showed macroscopic irregularities, and to some extent in those parts where symptoms could not yet be distinguished. The abnormal condition in the phloem could first be recognized by the denser appearance of contents of sieve tubes and companion cells in certain areas; then followed a "necrosis, collapse, discoloration, and often, in the end, development of new, irregularly formed, wound-healing cells in these areas."

Rawlins[3] observed a degeneration of the pericycle cells and "several rows of adjacent cells" in root tips from curly-top-diseased sugar beets.

MATERIAL AND METHODS

Material for study consisted of sugar beets of the morphologically uniform strain 2705g24—very susceptible to curly top—developed by W. W. Tracy of the United States Department of Agriculture; and of the commercial strain, Rabbethge and Giesecke's "Old Type." Part of the plants were grown in pots in the greenhouse; the others, in a field plot.

[3] Rawlins, T. E. Cytology and other studies of curly-top disease of the sugar beet. Unpublished thesis. Library, University of California, Berkeley, Calif. 1926.

To obtain diseased material, virus-carrying males of the beet leaf hopper, *Eutettix tenellus,* were caged upon individual leaves, usually 5 leaf hoppers to a plant, and were removed, as a rule, 72 hours later. The beets in the field plot also were inoculated artificially by means of leaf hoppers reared in cages. In the field the insects were not caged upon individual plants but were set free. Those used for inoculation were fed beforehand on sugar-beet plants affected by curly top of a form that Carsner (14) designates as severe. The experimental plants received no other special treatment. Both greenhouse and field-plot beets were entirely free from sugar-beet mosaic.

The material was killed and fixed for 48 hours in Karpechenko's weak chromo-acetic-formalin solution made up as follows: solution A: 65 cc water, 10 cc glacial acetic acid, 1 gram chromic acid; solution B: 65 cc water, 10 cc commercial formalin (40 per cent stock solution). Equal quantities of solutions A and B were mixed immediately before placing the material in the killing fluid. For the first 12 hours the material was kept in cold storage at 45° F. Butyl alcohol was used for dehydration and clearing. The material was left 24 hours in each change of the ethyl-butyl-alcohol mixtures—longer than suggested by Zirkle (71). The sections were cut 10 microns in thickness and stained with Heidenhain's iron-alum haematoxylin and with a counter stain of light green in clove oil. Fresh material was sectioned by hand and observed in either tap water or glycerine.

PATHOLOGIC CHANGES IN THE PHLOEM AND PERICYCLE REGION

Structure of the phloem and pericycle region in healthy leaves.—Necrosis, a constant symptom of beets affected by curly top, involves the phloem and the region external to it—the bundle cap. This fact raises the question as to the composition of the bundle cap and its relation, if any, to the phloem.

According to de Vries (70), a vascular bundle in a beet leaf consists of the following tissues (Figs. 2, A and 10, A): on the ventral side of the leaf, a small bundle sheath consisting of long, thick-wall cells; then primary xylem, a layer of secondary xylem, cambium, phloem, and finally a "bast sickle" composed of cells whose walls are collenchymatously thickened. These "bast" cells have oblique radial walls and, frequently, thin transverse ones. The last layer of cortical cells, that adjoining the vascular bundles, is a starch sheath, which, in young leaves, contains abundant starch. For all the bundles, there is usually one common starch sheath, the cells of which are smaller than those of the adjoining parenchyma of the cortex. No intercellular spaces occur between the starch sheath and the cells of the bundle cap. The midrib and larger lateral veins form ridges

protruding from the surface of the leaf blade, particularly on the dorsal side. These projections have a layer of collenchyma on both the ventral and the dorsal surfaces of the leaf (Fig. 10, A). In the petiole the collenchyma is confined to the ribs.

Artschwager (1) designates the bundle cap at the phloem end, as well as that at the xylem end of a bundle, as a sclerenchyma cap, but does not describe it in detail.

Structures known in botanical literature as "bundle caps" consist of fibrous cells and are commonly associated with vascular bundles in leaves. According to Fitting (28, p. 88), bundle sheaths, a special type of which is represented by bundle caps, do not belong to the vascular tissue. They may be of pericyclic or cortical origin (26, p. 256). Sometimes, however, sheaths may be assigned to the bundle itself (3, p. 318). The occurrence of sclerenchymatous pericycle is characteristic of Chenopodiaceae, although the sclerenchyma is frequently only weakly developed (60, p. 662).

This investigation showed that the bundle cap in beet leaves rarely exhibits lignification. Lignified cells in the cap have been found most frequently in the large bundles at the base of the petiole, but occasionally also in the midrib and the large lateral veins. Lignification, if it occurs at all, usually is confined to the outer layer of the cap, although lignified cells are sometimes present in the second and third layers. Cap cells, showing lignin reaction with phloroglucin and HCl, have distinct tertiary thickenings and oblique transverse walls. They appear as sclerenchymatous fibers; and their position, adjacent to the starch sheath, indicates a pericyclic origin.

The major portion of the bundle cap consists of several layers of cells with comparatively thick cellulose walls. The thickenings, being pronounced in the corners, give the tissue the appearance of collenchyma, as de Vries (70) has indicated. This tissue merges imperceptibly with that of the functioning phloem (Figs. 2, A and 4, A); nor is there any line of demarcation between these cells and those of the pericycle, which may later become lignified. A sharp line of demarcation appears, however, between the pericycle cells and the starch sheath (Figs. 2, A; 2, B; 10, A), cells of the latter having much thinner walls and containing starch. In longitudinal sections, the starch-sheath cells are much shorter than those of the bundle cap.

The pericycle does not form a massive tissue in the leaves. Very young bundles contain, adjacent to the starch-sheath mother cells, a row of cells that may be interpreted as pericycle. Then follows the primary phloem, whose first mature sieve tube may be readily discerned in transverse sections because it is pentagonal or hexagonal in outline and appears optically empty in imbedded material. In the following stages of bundle develop-

ment, radial divisions occur in the pericycle, permitting lateral expansion. Then the cells enlarge, and their walls become thicker. The changes occurring in the primary phloem involve obliteration of sieve tubes and probably of their companion cells. The parenchyma cells of the primary phloem enlarge like those of the pericycle, their walls becoming thicker, particularly in the corners, where remnants of obliterated elements are discerned. In larger bundles a few tangential divisions occur in the pericycle, especially in the median bundle; but on the average not more than one row of cells may be considered as pericycle, because traces of obliterated elements of primary phloem are frequently discernible, even in older bundles, one row of cells removed from the starch sheath.

As obliteration in primary phloem progresses, the cambium forms a secondary phloem. A considerable amount of secondary growth occurs in the petioles, midribs, and larger lateral veins of beet leaves. Obliteration takes place also in the secondary phloem (Figs. 2, A; 4, A; 7).

Obliteration in phloem of healthy leaves.—Obliteration of phloem elements is a normal process in many plants having secondary growth. In angiosperms, the sieve tubes, companion cells, and cambiform cells are reported to be subject to obliteration (8, 64). In gymnosperms the sieve tubes and closely associated ray and parenchyma cells become functionless and collapse (64). Seeliger (53) finds both sieve tubes and companion cells obliterated in the sugar-beet root. The obvious changes and loss of contents have been assumed to be primary causes in the process of obliteration, pressure exerted by the surrounding living elements being only a secondary factor (8, 3).

In sugar-beet leaves, obliteration apparently involves both sieve tubes and companion cells. In sieve tubes, various stages of the process have been clearly observed, making possible an interpretation of the origin of the bundle cap.

Figure 1 shows obliteration of phloem elements in a petiole of a healthy, young sugar-beet leaf. Figure 1, A, represents a section through a region of the functioning secondary phloem. Within the phloem, the sieve tubes may be recognized by scarcity of contents and by the presence of structures known as sieve-tube plastids. The latter, although typical of sieve tubes of most plants, have not been found in any other type of cells. They have been described as starch grains by Briosi (10) and Lecomte (40) and as leucoplasts by Strasburger (64), who states that they produce a certain kind of starch that stains wine-red with iodine. In beets these bodies appear spherical, stain as protoplasmic structures with Heidenhain's iron-alum haematoxylin, and brown with iodine. They usually are aggregated near the sieve plates. The companion cells may be recognized by their

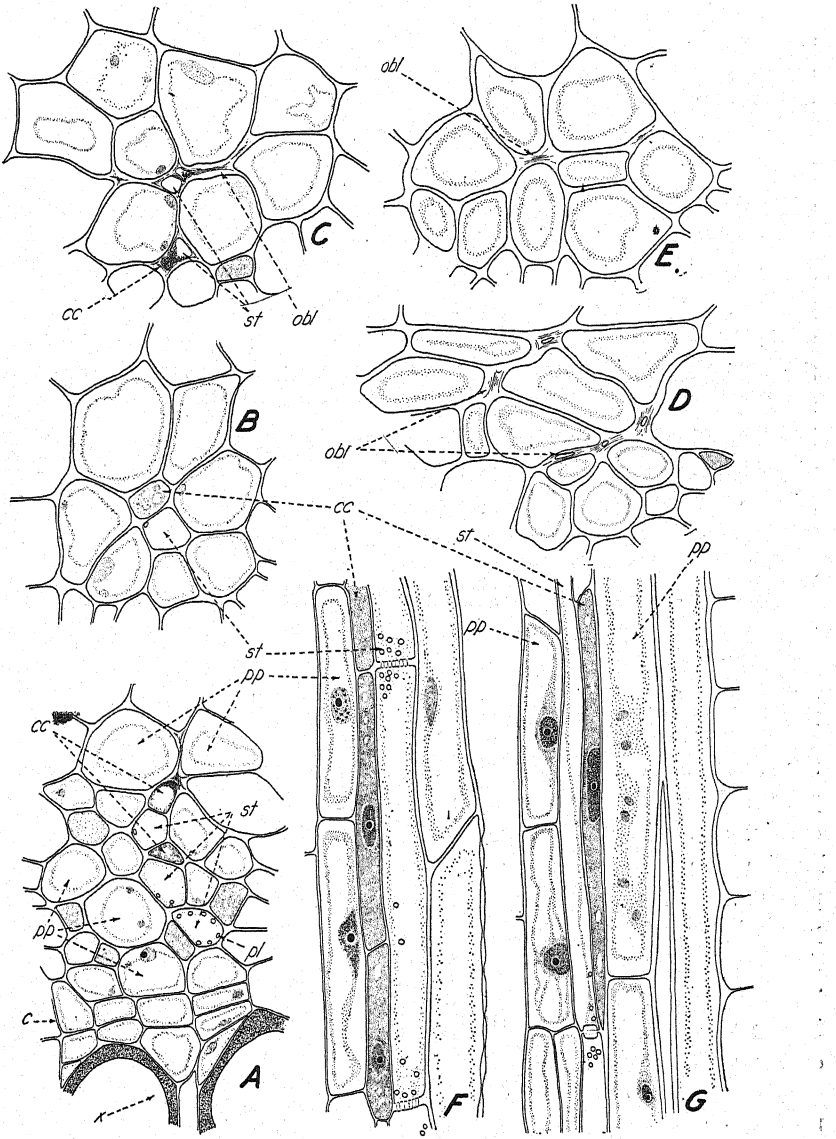


FIG. 1. Transverse (A–E) and longitudinal (F–G) sections of healthy phloem and bundle cap of petioles, showing obliteration of phloem elements. ×585. A and F. Functioning secondary phloem. B. Sieve tube and companion cell surrounded by enlarging phloem parenchyma cells. C. Early stage of obliteration. D and E. Advanced stages of obliteration. G. Early stage of obliteration; lumen of sieve tube decreasing, callus upon sieve plate increasing. Throughout figure: *st*, sieve tube; *cc*, companion cell; *pl*, sieve-tube plastid; *pp*, phloem parenchyma cell; *c*, cambium; *x*, xylem; *obl*, obliterated phloem elements.

position with respect to the sieve tubes and by their dense cytoplasm. In cross-section, the parenchyma cells of the functioning phloem are usually as large as the sieve tubes or larger; they contain a parietal layer of cytoplasm and some chloroplasts. Figure 1, B, shows a sieve tube and a companion cell surrounded by enlarging parenchyma cells. Figure 1, C, illustrates an early stage in the obliteration of sieve tubes and apparently of their companion cells. Some change has taken place, the contents of these elements being less abundant and the lumina of the cells having decreased in size. Figure 1, D, is drawn from the region of the primary phloem. Of the obliterated elements there remain only thickenings in walls between phloem parenchyma cells, with small, narrow slits indicating the position of the former lumina of the obliterated cells. In figure 1, E, no slits can be recognized in the region of obliteration. Figure 1, F, shows, in a longitudinal section, phloem parenchyma cells, a sieve tube with plastids, and companion cells from the region of the functioning phloem. Figure 1, G, represents an early stage in the obliteration of a sieve tube. The latter has become narrower, and the callus upon the sieve plate is more conspicuous than in figure 1, F.

The data on the structure of the phloem and bundle cap—the region that undergoes necrosis in curly-top-diseased sugar-beet leaves—may be summarized as follows. It is composed of the pericycle, the primary phloem, and the secondary phloem. In older leaves the primary phloem is represented only by phloem parenchyma, the sieve tubes and probably the companion cells having become obliterated. The primary phloem parenchyma and the pericycle cannot be distinguished readily, for they consist of similar cells and together, form a structure that appears as a bundle cap of collenchymatous elements located outside the secondary phloem. After the obliteration of sieve tubes in the secondary phloem the parenchyma of this phloem also becomes a part of the bundle cap. When Artschwager (1) states that "above the phloem is a large sclerenchyma "cap" and that "the phloem forms a narrow zone," he apparently includes in the "sclerenchyma cap" the parenchyma of the primary and of part of the secondary phloem.

Localization of necrosis in the phloem and pericycle.—Necrosis induced by curly top may sometimes be observed mainly in the secondary phloem (Fig. 2, B), may involve a portion of the cap (Fig. 3, A), or may transform the whole bundle cap into a large necrotic pocket (Fig. 2, C). In other words, in some bundles degeneration occurs chiefly in the secondary phloem (Fig. 2, B), while in others the primary phloem and pericycle regions are necrotic (Figs. 2, C, and 3, A). In young leaves, developed some time after infection of the plant, the bundle cap is entirely deformed

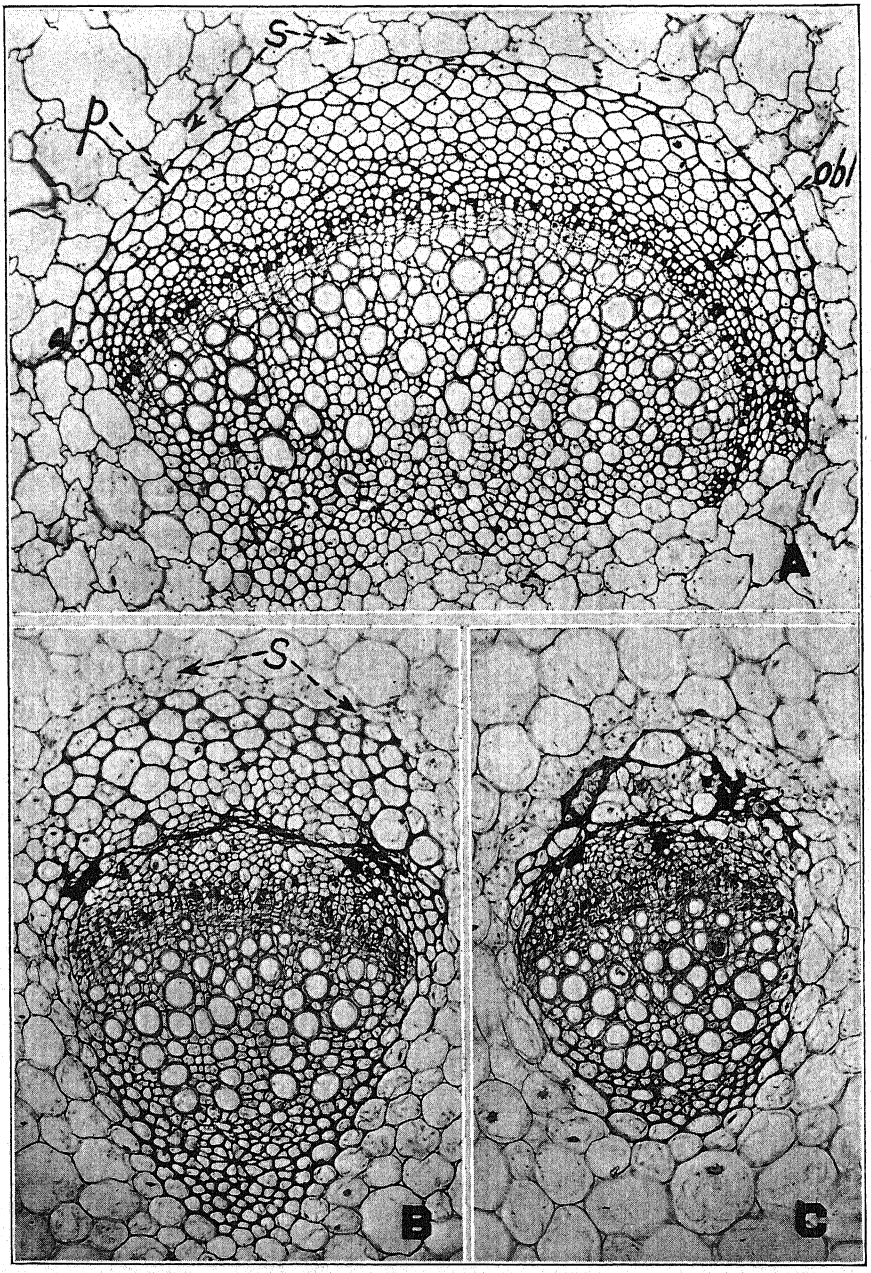


FIG. 2. Transverse sections of vascular bundles from petioles. ×125. A. Healthy. B. Diseased, showing necrosis in the secondary phloem. C. Diseased, showing complete destruction of bundle cap. Throughout figure. *p*, pericycle; *s*, starch sheath, *obl*, obliterated phloem elements.

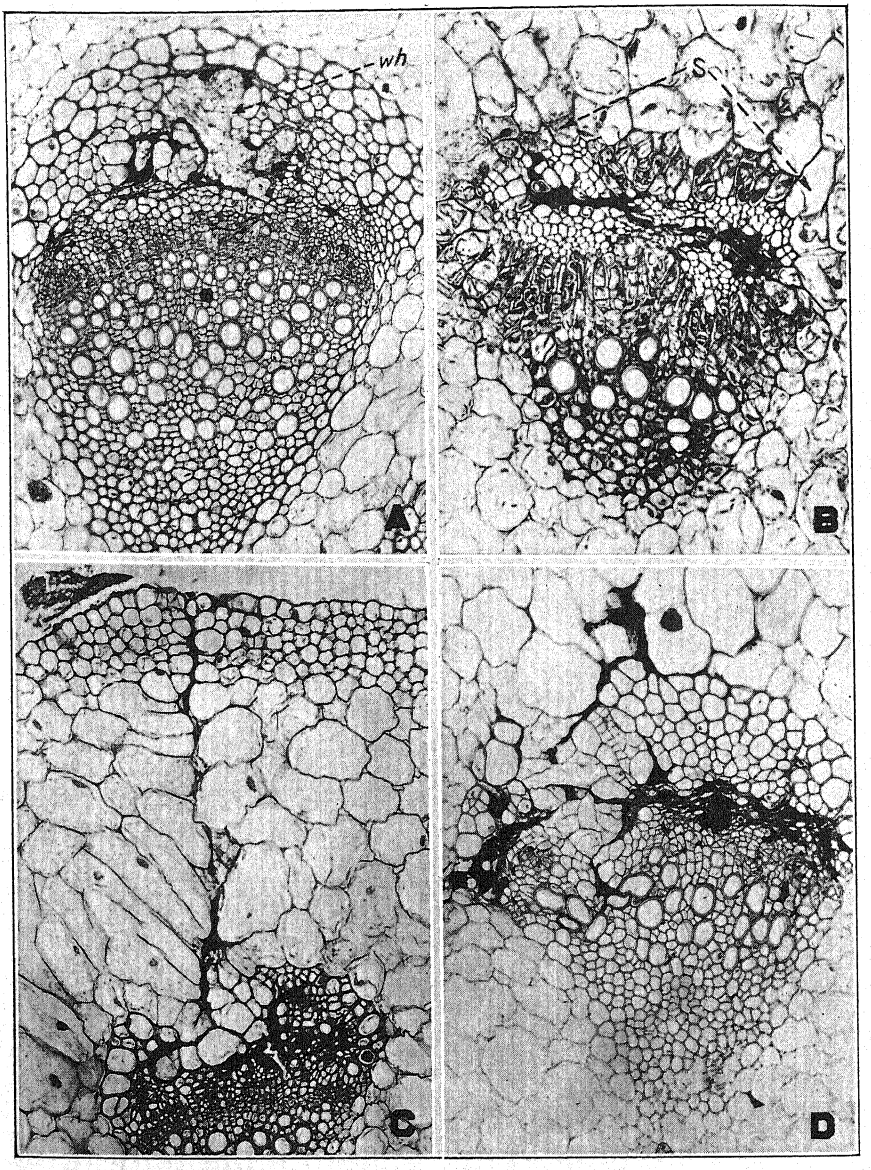


FIG. 3. Transverse sections of vascular bundles from petioles of a diseased plant. A. Bundle from mature leaf. The cap is partly destroyed. Cell proliferations, resembling wound-healing tissue (*wh*), have filled the cavity that resulted from collapse of certain cells. ×98. B. Bundle from young leaf. Cap deformed because of early hyperplasia in phloem and pericycle region. Line of demarcation between pericycle and starch sheath (*s*) very distinct. Necrotic area surrounded by hyperplastic tissue whose cell contents are scarce. ×200. C. Exudation of material from necrotic phloem to the exterior. Cortical cells along the path of the exudate show hypertrophy. ×98. D. Exudation of material from necrotic phloem into the xylem. Two vessels are partly crushed. ×98.

(Fig. 3, B), because the primary phloem and the pericycle have degenerated.

Necrosis has not been observed in any other tissue. In some cases material originating in the necrotic phloem may escape into the xylem, passing through the intercellular spaces and even filling some vessels (Fig. 3, D). It also may escape into the intercellular spaces in the cortex and exude to the exterior of the leaf (Fig. 3, C); but necrosis is apparent in neither the xylem nor the cortex. On the contrary, the parenchyma cells along the path of the exudate are stimulated to growth (Figs. 3, C, and D).

Necrosis of the phloem, as induced by curly top cannot be confused with normal obliteration. Necrotic areas in diseased plants are quite large (Fig. 4, B) as compared with the few cells involved in obliteration (Figs. 2, A, and 4, A). In the areas of obliteration, relatively inconspicuous thickenings of walls indicate the former position of the obliterated elements, whereas in the necrotic regions, collapsed walls form conspicuous layers, wavy and irregular in outline, and frequently showing traces of lumina of the dead cells. In necrotic areas accumulates a large amount of amorphous material, which stains deeply with coal-tar and haematoxylin dyes. No such accumulations occur in the region of obliteration. In fresh sections, the material in necrotic areas appears brown.

Hypertrophy and hyperplasia in the phloem.—Further studies must precede the interpretation of the stages in the degeneration of the phloem of beets affected by curly top. Preliminary studies indicate that, before the formation of lesions, hypertrophy and hyperplasia occur in the pericycle and phloem. Some cells divide in any plane; others hypertrophy without dividing, while their contents disintegrate.

In later stages certain cells collapse, thereby causing the appearance of lesions. In very young leaves collapse of cells does not result in a formation of cavities. The actively dividing cells probably exert a pressure upon the dying cells, hastening their collapse, and, afterward, form a small-cell, irregular tissue that surrounds the necrotic areas. These cells have dense cytoplasm at first; later, their contents become so scant that they stand out conspicuously among other tissue (Fig. 3, B).

In older leaves, necrosis leads to a formation of conspicuous cavities. The dark-staining material, which is present in lesions of recent origin, disappears later; and the cavities are closed by an irregular tissue resembling callus (Fig. 3, A). This tissue, having thin walls, may be readily differentiated from the hyperplastic tissue, just described for young leaves (Fig. 3, B). Evidently, both these types of tissue are a result of hyperplasia, but one begins to form before, the other after, the collapse of cells.

It would seem that the early hyperplasia is a response to some disturbance caused by the presence of the virus in the phloem, but the prolifera-

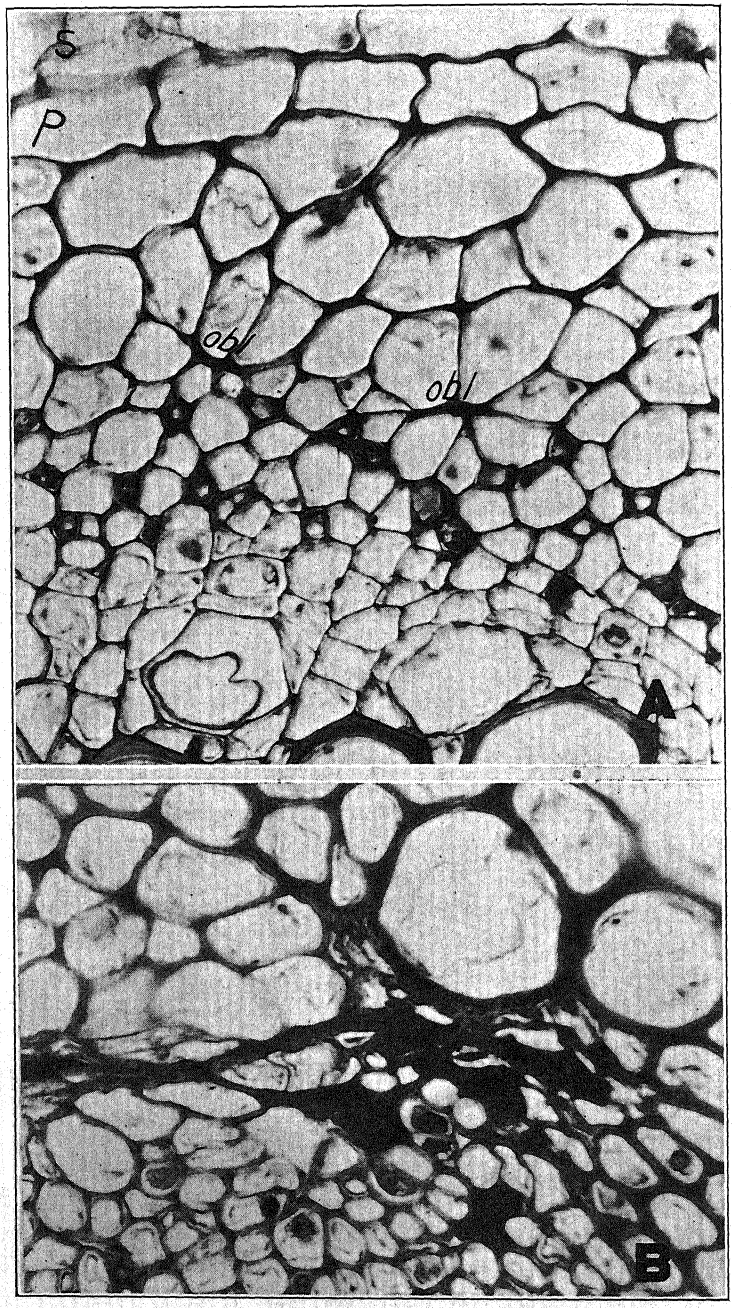


FIG. 4. Transverse sections of phloem of petiole. ×585. A. Healthy, showing obliterated phloem elements (*obl*), starch sheath (*s*), and pericycle (*p*). B. Diseased, showing collapsed walls and accumulation of deeply staining material in necrotic region.

tions connected with the lesions resemble wound-repair reactions. According to Küster (38, p. 76), cavities in a plant body, which result from death of cells, are similar to wounds, inasmuch as their presence induces the adjacent living cells to growth and divisions. The cavities in the phloem of curly-top diseased leaves resemble wounds, and the hyperplastic tissue, which closes these cavities, may conceivably have an origin similar to that of a wound-healing or callus tissue. Even if no conspicuous cavities are formed, hypertrophy and hyperplasia in tissues adjacent to necrotic areas may be comparable to repair or wound-healing reactions.

The phloem-parenchyma starch sheath and the cortical parenchyma have been observed to participate in the hypertrophic and hyperplastic processes in regions adjacent to the lesions in curly top. When necrosis does not involve the whole bundle cap, phloem parenchyma cells give rise to proliferations. This is the more frequent condition. When the whole cap is disrupted, the starch sheath cells may become meristematic and produce hyperplastic tissue. Other parenchyma cells may hypertrophy and divide near the lesions.

Figures 5 and 6 illustrate hypertrophy and hyperplasia in connection with the lesions in the phloem of a diseased leaf. Figures 5, A, and B, show callus-like tissue formed by phloem parenchyma. The intact starch sheath may be seen on the right, next to the lesion. In figure 5, A, the proliferated tissue is very irregular, while in figure 5, B, the cells appear to form a cambium. Figure 6 illustrates phloem parenchyma cells that have divided and filled a cavity. Figures 5, C, and D, show hypertrophy and hyperplasia of starch sheath cells. In figure 5, C, starch sheath cells still contain abundant plastids, but the cells have already elongated in a direction perpendicular to the surface of the cavity; the first cross walls have been laid down parallel to the surface of the cavity. In figure 5, D, the plastids have disappeared and the starch sheath cells have given rise to a small-cell, thin-wall tissue.

The filling of spaces by tissue produced through the division of neighboring cells is common in plants, even in their normal ontogeny. Wounding, whether external or internal, stimulates the adjacent living cells to growth and division (11, 38). The phenomena involved in this stimulation are not yet well understood. Some investigators would connect them with the physico-chemical changes caused by wounding or attribute them to the action of certain materials produced by necroses (38).

All living elements, if adjacent to wounds, may be stimulated to growth and division (38). Even cells having thick cellulose (collenchyma) or lignified (xylem-parenchyma) walls may participate in callus formation (19, 52), in which the cambium and the ground parenchyma are particularly efficient (38).

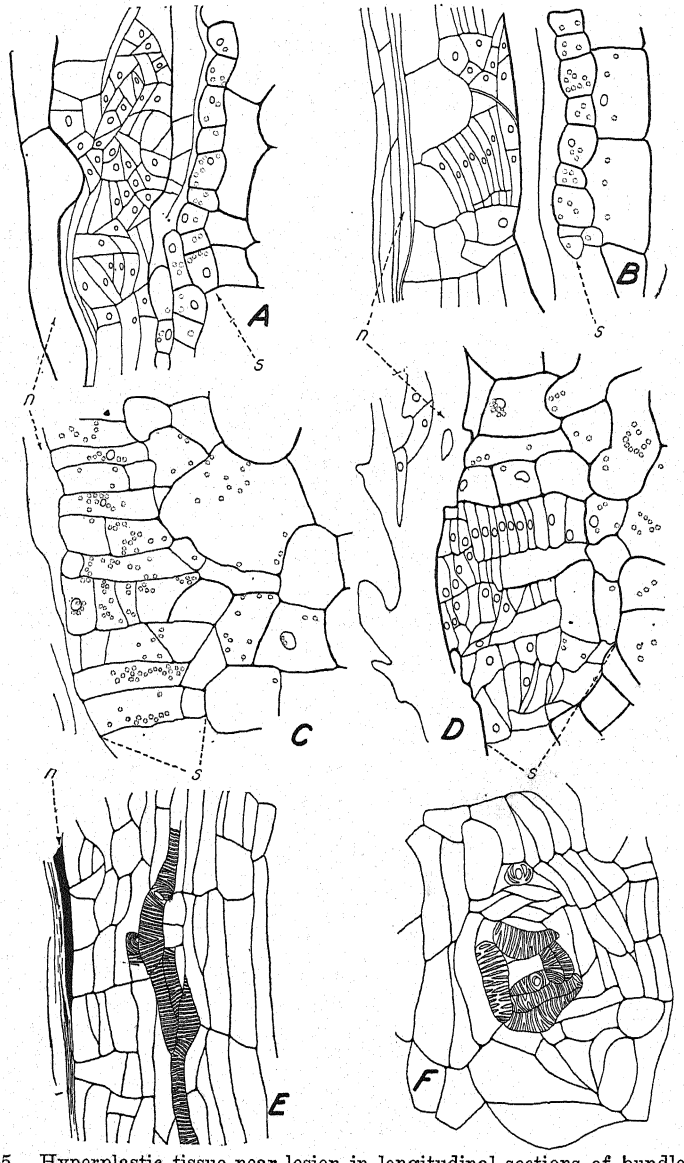


FIG. 5. Hyperplastic tissue near lesion in longitudinal sections of bundle cap from diseased leaf. A–E. ×147; F, ×239. A. Hyperplastic tissue formed by bundle-cap cells adjacent to necrotic area (*n*); starch sheath (*s*) intact; proliferated tissue irregular. B. As in A, but shows formation of cambium in proliferated tissue. C. Hypertrophy and early hyperplasia of starch-sheath cells (*s*) near necrotic region (*n*). D. Advanced hyperplasia of starch-sheath cells (*s*). E. Continuous row of xylem elements differentiated in hyperplastic tissue. F. Isolated ring-like group of xylem elements differentiated in the hyperplastic tissue.

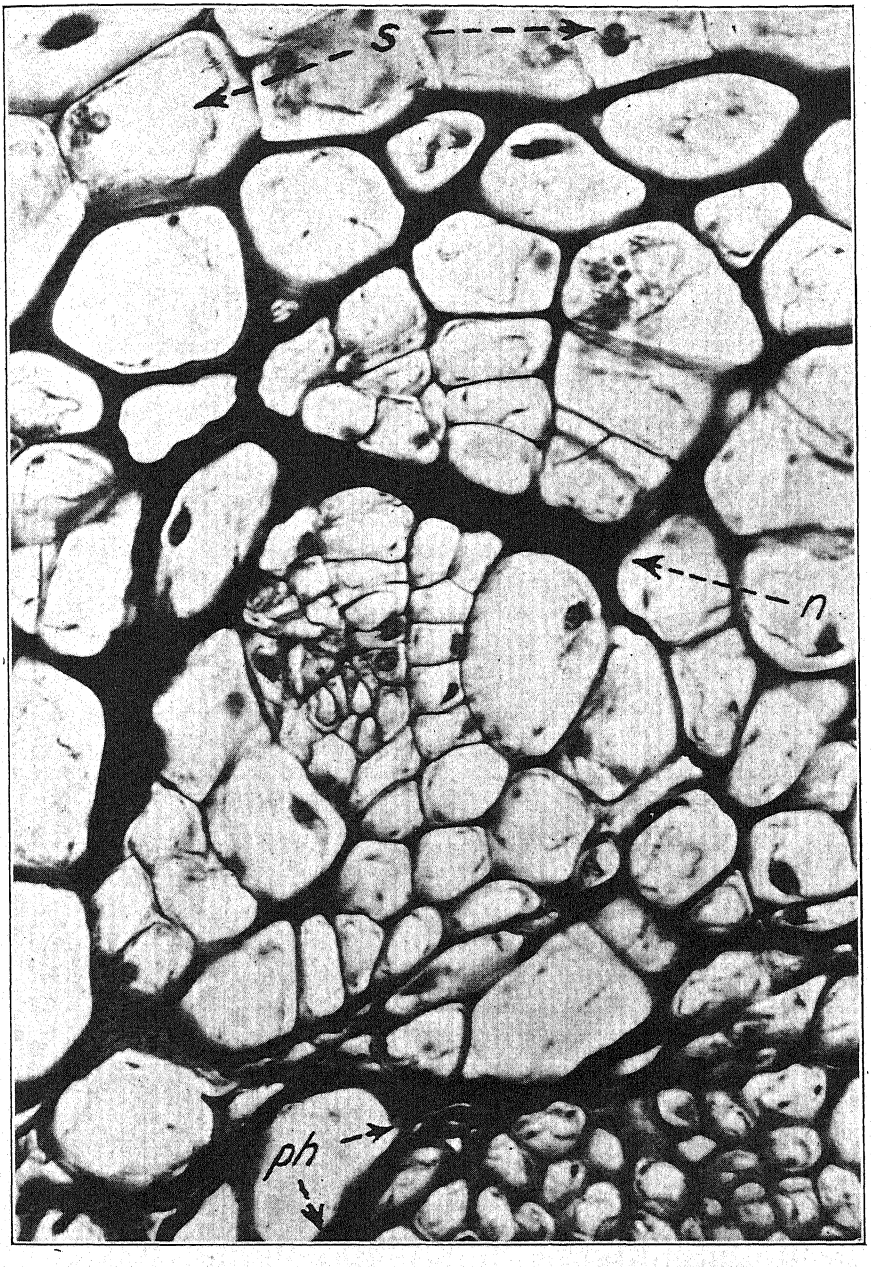


FIG. 6. Transverse section of diseased bundle cap, showing early stage of differentiation of hyperplastic tissue near lesion into vascular tissue. ×725. *ph*, phloem; *n*, necrotic area; *s*, starch sheath.

Phloem parenchyma in the sugar beet, being normally very active in producing meristematic tissue (53), may be readily stimulated to growth and division in the neighborhood of lesions in curly-top plants. The same is true of the pericycle, which undoubtedly participates in the filling of the cavities in diseased beet leaves. The starch sheath and cortex consist of parenchymatous cells, generally active in producing callus near wounds.

Frequently cork, instead of callus, is formed around wounds or lesions (11, 38, 42), but this has not been observed in curly-top diseased sugar beet leaves.

Secondary vascular tissue in the bundle cap of healthy leaves.—In sugar-beet leaves, severely affected by curly top, xylem elements frequently differentiate in the hyperplastic tissue in the degenerated bundle cap. This fact raises the question as to the possible relation of this xylem to the secondary cambium and vascular tissue normally produced in the pericycle and primary phloem region in roots and flowering stalks of healthy beets.

The sugar beet, like other members of the Chenopodiaceae, shows anomalous secondary growth caused by the function of supernumerary rings of secondary cambium. In the root, according to Seeliger (53), the first ring of cambium is formed in a normal way between the primary xylem and phloem. When this cambium begins to function, certain primary phloem parenchyma cells enlarge and divide, while the sieve tubes and their companion cells become obliterated. From primary-phloem cells is differentiated the first supernumerary ring of secondary cambium, which forms xylem elements and parenchyma centripetally and phloem elements and parenchyma centrifugally. Before the first phloem elements of this ring are differentiated, a cell is cut off centrifugally from the cambium initial, which becomes the initial for the second supernumerary ring of vascular tissue. This initial, in its turn, gives rise to the initial of the third supernumerary ring, and so forth. The rings of vascular tissue arising from the supernumerary cambiums appear in a cross-section of a beet root as concentric annular rings. In certain regions of the plant, the pericycle also participates in forming the initials of the secondary cambiums. In the root these initials arise mainly in the phloem parenchyma, as just described; in the upper part of the hypocotyl in the pericycle; and in the lower hypocotyl in both the phloem parenchyma and the pericycle. Although such is the usual situation, exceptions are very common: the pericycle and the primary phloem-parenchyma cells may substitute for one another very readily in all layers of the root and hypocotyl during the formation of meristematic tissue. Cambium may arise occasionally in the parenchyma cells of the secondary phloem. The pericycle and phloem-parenchyma cells have an unusual capacity for becoming meristematic;

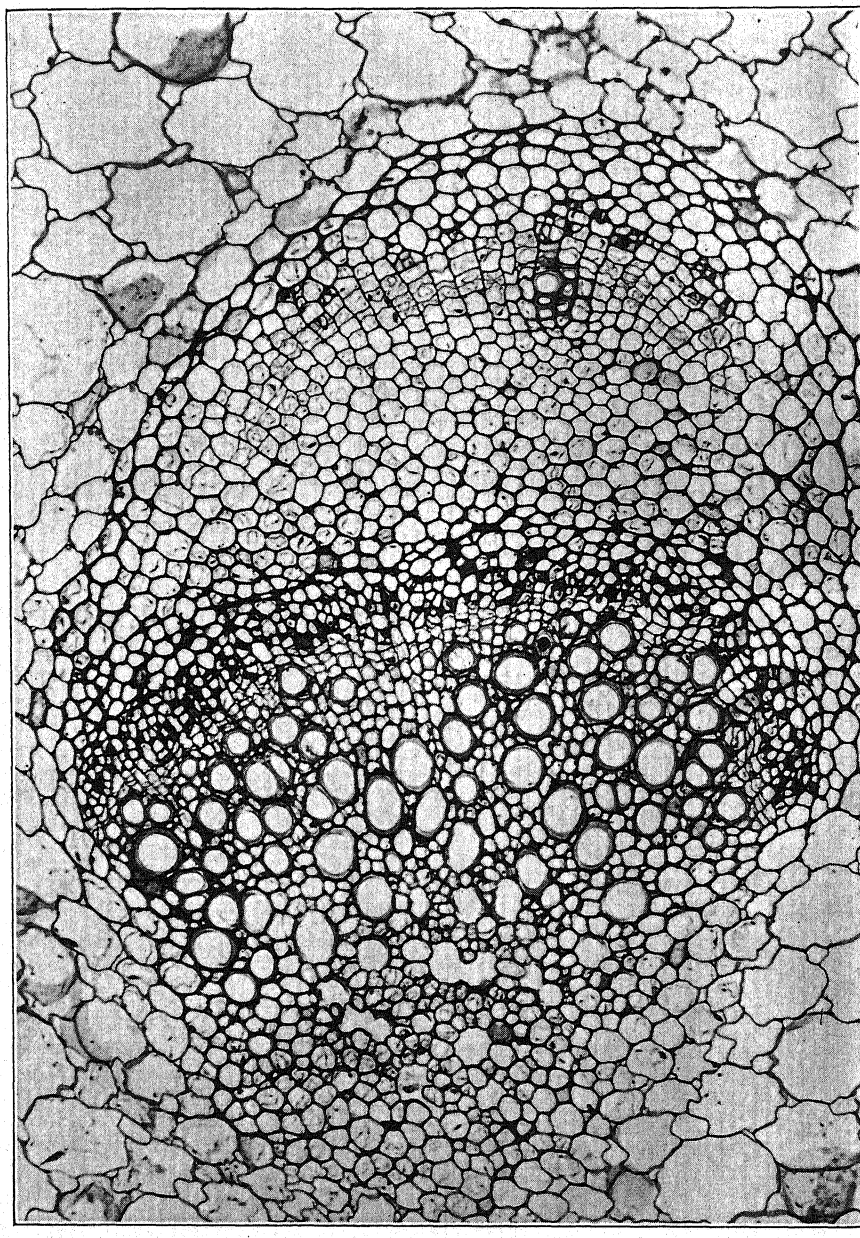


FIG. 7. Transverse section of a healthy bundle, showing formation of secondary cambium in bundle cap. First phloem and xylem elements have differentiated from this cambium. ×180.

being very slightly specialized, they may give rise to all types of cells that occur in a beet plant.

Artschwager (2) reported that in the floral axis the secondary cambium arises in the pericycle.

In this study, a large number of healthy young and old leaves, including those taken from flowering stalks, showed, upon examination, that secondary cambium forms to a limited extent in sugar beet leaves. Figure 2, A, represents a bundle without a secondary cambium, and figure 7 shows one, at the base of the petiole, in which a secondary cambium has arisen in the cap; from this cambium some phloem and several xylem elements have matured. Frequently, several of the larger bundles at the base of an old leaf show secondary vascular tissue in the cap.

In petioles the secondary cambium and the vascular tissue arising from it have been seen to form in the pericycle, but more frequently in the parenchyma of the primary phloem. They have been noted only in a short region at the base of the petiole. In cases studied, no such secondary vascular tissue was found at the insertion of the lamina or in the midrib or lateral veins.

Differentiation of xylem in the degenerated bundle cap of diseased leaves.—In the diseased leaves xylem differentiation in the cap occurs not only in the petiole, but in the midrib and lateral veins. This xylem is abnormal in its distribution and structure. It usually shows irregular distribution in both transverse and longitudinal sections (Figs. 8, A, and B). Cells of the callus-like tissue either differentiate directly into xylem elements or behave as cambium, forming radial rows of longitudinally elongated cells that mature into xylem elements. In the first case the xylem shows a very irregular distribution, frequently forming rings of xylem elements (Fig. 5, F) like those described by Küster (38) in the case of callus in Beta. These groups may have no connection with the rest of the abnormal xylem. In the second case, the abnormal xylem runs parallel to the normal and appears more regular (Fig. 5, E). Figure 6 shows an early stage in the development of vascular tissue in proliferated tissue near a lesion.

The normal xylem arising from a secondary cambium shows scalariform, scalariform-reticulate, and pitted elements and lignified fibers with unsymmetrical pits having narrow mouths. The abnormal xylem contains no fibers and consists of spiral, scalariform, and scalariform-reticulate elements.

Transverse and longitudinal sections of diseased leaves in no case revealed a connection between the xylem in the hyperplastic tissue and the normal xylem.

Leaves cleared in lactic acid (method by Simpson, 56) showed that the abnormal xylem may run parallel to the normal xylem through the petiole

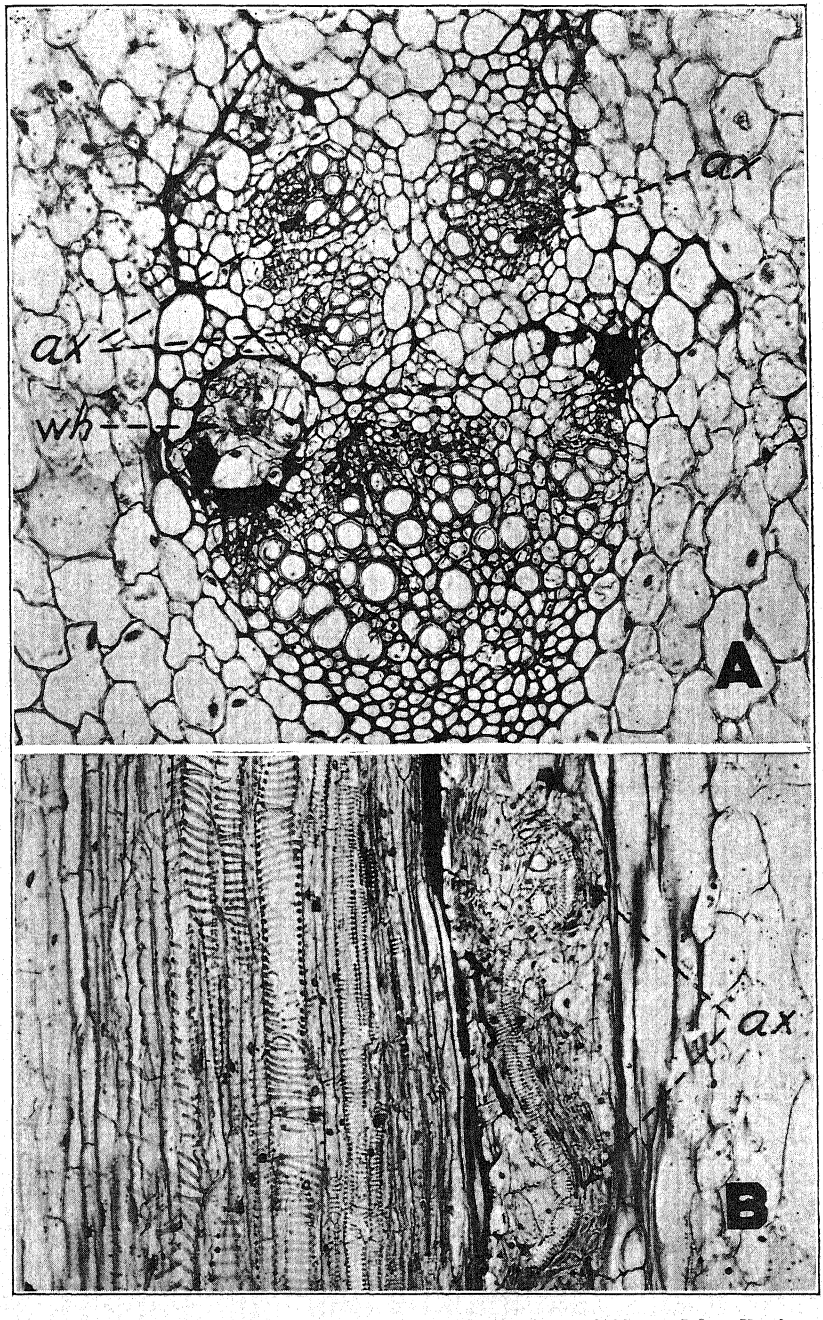


FIG. 8. Transverse (A) and longitudinal (B) sections of diseased bundle from the petiole, showing abnormal xylem (*ax*) and hyperplastic tissue near lesion (*wh*) in the bundle-cap region. ×174.

and midrib and into the lateral veins without forming any anastomoses with the normal xylem. This method also made it possible to distinguish islands of abnormal xylem that have no connection with the rest of the abnormal xylem.

The absence of connection between normal and abnormal xylem was also ascertained by introducing a stain into the xylem. This work was done under a dissecting binocular microscope, magnification × 50, a concentrated solution of acid fuchsin in tap water being used. The studies were made on leaves severed from plants. At first a strand of abnormal xylem was freed with a dissecting scalpel from surrounding tissues in a small area ½ in. above the base of the petiole. A drop of the stain was then placed upon the freed portion of the strand, which was being supported upon a scalpel. With a second scalpel the strand was then cut under the fuchsin solution. After 5 minutes or thereabouts, the cortical tissue was removed above the vascular strand all along its course, and the distribution of the stain was determined. It proved to remain confined to the abnormal xylem, which appeared as a red line upon the white background of the normal xylem underneath. The abnormal xylem itself was continuous, and the stain spread from the base of the petiole into the abnormal xylem of the lateral veins. In several cases tracheal tubes leading along the midvein would suddenly terminate in a ring-like group of elements; from this point on, only one tube would continue into the lateral vein. Introduction of the stain into the normal xylem only was also tried, the strand of abnormal xylem being in this case removed for a distance of approximately an inch at the base of the petiole. The latter was then immersed in acid fuchsin solution, and a small section cut off at the base. The stain entered the normal xylem but was not found in the clipped strand of abnormal xylem. The latter appeared as a white line upon the red background of stained normal xylem. The stain, when introduced into an individual normal xylem strand, was distributed through all the other normal strands but did not enter the abnormal xylem.

Whether or not phloem differentiates along with abnormal xylem has not been determined. The adjacent tissue often resembles phloem in cross-section, but no sieve tubes were definitely recognized in longitudinal sections.

The formation of xylem in the hyperplastic tissue of curly-top leaves is not an extraordinary phenomenon. Xylem and phloem commonly differentiate in callus (38), and, moreover, the tissues of Beta are readily stimulated by wounding to produce cambium and vascular tissue (69).

Exudate from necrotic phloem.—The previously mentioned exudate from diseased leaves was tested according to Flückiger as quoted by Tun-

mann (66, p. 184). Only traces of reducing sugars were observed. The Cu_2O precipitate was, however, abundant after inversion with HCl, a fact indicating that sucrose is the predominant carbohydrate constituent of the exudate. Similar results were observed by Carsner and Stahl (15), employing Fehling's test.

The microchemical tests on the exudate thus far made are not a sufficient basis for any conclusions regarding the proportions of carbohydrate components of the exudate. The fact, however, that the latter contains sugar apparently indicates that it is derived from the phloem, although, according to Ruhland (47), the sugar is translocated in beet leaves mainly in the form of invert sugar. The anatomical studies also show that the exudate may be traced back to the phloem region, from which it escapes through the intercellular spaces (Figs. 3, C, and D). The exudate is probably the material translocated in the phloem. According to Crafts (18) the material in healthy phloem is under high pressure, but remains confined to this tissue because of the presence of limiting layers (cambium on one side and the starch sheath on the other). In view of this theory, the suggestion might be made that necrosis of the phloem so affects the limiting layers that they cannot resist the pressure existing in the phloem, and the translocated material escapes into the xylem or to the exterior. On the other hand, there is a possibility that the pressure existing in the phloem is increased in diseased leaves (15) and that the material is forced into the intercellular spaces of surrounding tissues.

PATHOLOGIC CHANGES IN TISSUES OTHER THAN THE PHLOEM

Clearing of the veins.—At this point, a few words are necessary concerning the appropriateness of the expressions *clearing of the veins* and *transparent venation.* The term *vein* in botanical literature is used rather loosely. It is commonly applied both to the vascular tissue and to the tissues in which the vascular strands are imbedded and which, in the case of larger strands, form protrusions and ridges. In a more exact terminology, according to de Bary (3, p. 299), the term *vein, nerve,* or *rib* is applied only to the protrusion, that is, to the external formation of parts; while the *vascular bundle* or *strand* refers to the inner structure. The former loose usage of the term *vein,* being very convenient, has become established. There should be, therefore, no objection to the continued mention of clearing of veins, the more so as this expression describes a phenomenon that may be recognized externally. A substitution of *translucent* for *transparent* is to be recommended, because the veins in curly-top leaves do not permit objects to be seen through them (transparency), but they admit the passage of light (translucency) to a greater extent than the veins in healthy leaves.

Clearing of the veins has been described not only in curly-top sugar beets, but also in the case of plants affected by certain other virus diseases. In curly top, however, it appears not to be identical with clearing of the veins in some other diseases. In the case of crinkle-"A" mosaic disease of potato, the veins of the young leaves become bright yellow and necrotic (48, 49); in asters affected by yellows, only a slight yellowing appears along the veins (36). In curly-top beets, on the other hand, the veins become neither yellow nor necrotic, but appear translucent in transmitted light. This condition is not comparable to that termed "water soaked."

No investigator has given definite morphological and physiological description of clearing of veins. Kunkel (37) suggests that this symptom may result from a stimulation of cell growth in the phloem.

In curly-top leaves showing translucent veins, the small veinlets may be recognized in transmitted light. In a healthy leaf only the larger veins are somewhat translucent, because their protrusion consists of large cells containing few chloroplasts (Figs. 10, A, and 9, C), but the small veins are surrounded by mesophyll cells with a large number of chloroplasts.

Clearing of the veins is an early symptom and usually appears in all newly-developed leaves as long as the plant lives. Very soon, however, the translucent veins become swollen. In severely affected plants the translucent-venation stage is short, the veins in very young leaves becoming thickened and distorted. In less severely affected plants the swelling may be very slight or undiscernible with the naked eye. Blisters (55) resemble translucent venation except that the clearing and swelling are confined to isolated areas along the veins.

Changes in leaves that may be recognized at the stage of cleared veins involve both the phloem and the adjacent mesophyll. As previously mentioned, in the early stages of disease hypertrophy and hyperplasia occur in the phloem. These may be recognized in leaves showing translucent veins. The stimulation of cells to growth and division extends beyond the phloem, however, involving mesophyll cells adjacent to the bundle on the phloem side. These cells divide radially and become so tightly packed as to leave no intercellular spaces. The chloroplasts are few and pale, some showing irregular shape, which indicates a condition of degeneration. This stage is represented in figure 9, A. For comparison, a cross-section of a healthy leaf is represented in figure 9, B. The mesophyll here shows the same number and size of chloroplasts below and above the small bundle. Intercellular spaces are formed very early in the mesophyll of healthy leaves on both sides of the bundle, whereas, in the diseased leaf, such spaces are absent on the phloem side. This change occurs not only in cells adjacent to the phloem but extends to the epidermis on the dorsal side of the leaf

and laterally beyond the phloem region. In subsequent stages the cells near the phloem elongate radially and become distinctly larger than those farther removed.

Leaves showing clearing of veins were taken from 2 kinds of plants: those recently inoculated with the virus and just having developed trans-

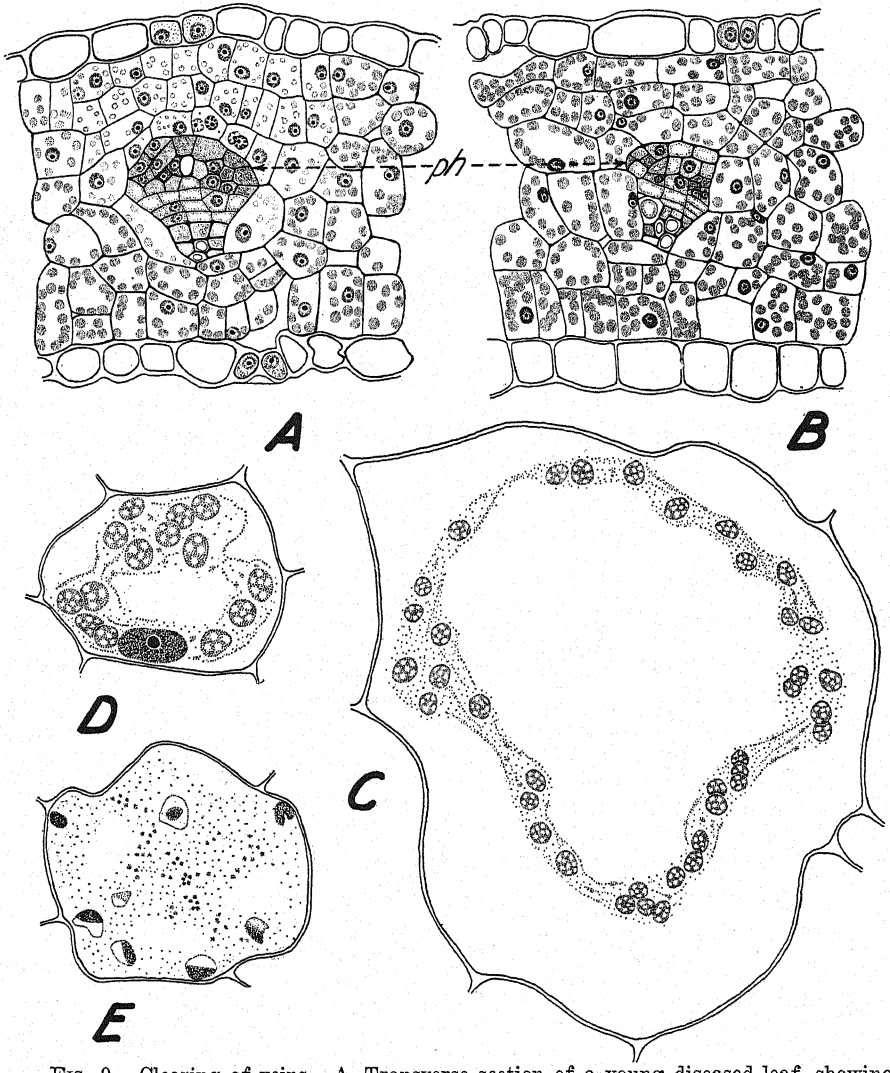


FIG. 9. Clearing of veins. A. Transverse section of a young diseased leaf, showing hyperplasia of cells and degeneration of chloroplasts in mesophyll adjacent to phloem (*ph*). ×450. B. Transverse section of a young healthy leaf. ×450. C. Parenchyma cell from a large vein of a young healthy leaf. ×1060. D. Healthy mesophyll cell from region adjacent to phloem of small bundle. ×1060. E. Diseased mesophyll cell from region adjacent to phloem of small bundle, showing degenerated chloroplasts. ×1060.

lucent veins or blisters; and those that had been infected for a long time but in which the youngest leaves showed pronounced translucent venation. The degeneration of chloroplasts was observed in both kinds of leaves.

In fresh sections appear degenerating pale green chloroplasts of irregular shape. Considerable hyaline granular material is present in cells where the chloroplasts have degenerated to such a stage that few or none are present in the cells. In fixed material the degenerating chloroplasts stain more weakly than normal ones, so that the regions about the phloem in stained sections appear pale. In such material the chloroplasts are of irregular shape and in many cases they appear as small bodies surrounded by a halo. The granular material also was recognized in stained sections. Figures 9, D, and 9, E, compare cells containing healthy and degenerated chloroplasts in stained sections. These cells were drawn from similar regions in the mesophyll near the phloem of a small vein in a healthy and a diseased leaf, respectively. Figure 9, C, shows a cell taken from a protrusion of a large vein. The cell is larger than the mesophyll cell in figure 9, D, but contains smaller chloroplasts, fewer in number per unit volume.

A marked feature of many of those cells showing degeneration of chloroplasts was an extremely rapid movement of small granules—too rapid to be accounted for by the protoplasmic streaming and apparently indicating some change in the structure of the cytoplasm. Dickson (20) observed a similar phenomenon in mesophyll cells of various plants affected by certain mosaics.

The degenerating cells were further characterized by the presence of numerous small vacuoles, as large as healthy chloroplasts or larger. Such vacuoles may occasionally be observed in healthy cells also, but much less frequently than in diseased. The vacuoles were not observed in the material embedded in paraffin, but appeared in sections treated with the killing solution (Karpechenko's weak) for a few minutes and then mounted in glycerine. In such material, the vacuoles could be made to show more distinctly by staining the sections with anilin blue. Numerous small granules, apparently, had accumulated at the surface of the vacuoles and, by absorbing blue stain, gave a sharp outline to the vacuoles. In fresh sections these granules appeared green.

The various phenomena described above have been observed also in the case of many other diseases. Translucent tissue with reduced intercellular space has been described in virus-diseased plants by several workers, among others by Dickson (20), Doolittle (21), Gardner (29) and Rand (46). The hypertrophy of green cells is usually accompanied by the disappearance of chloroplasts (38). Such cells may become totally depleted of chlorophyll, as in the case of intumescences (61).

Many virus diseases affect the chloroplasts and thus induce chloroses of various types. Some investigators report that the chloroplasts disintegrate (22, 27, 32, 46, 62); others attribute their reduction in size and number in certain mosaic diseases to inhibition of their development (9, 16, 17, 31, 35). In severe cases of certain diseases, the chloroplasts have been reported to disintegrate, while in milder cases of the same disease their development was only inhibited (20, 33). Apparently, both hypoplasia and disintegration of chloroplasts may take place in virus diseased plants, depending upon the stage of development at which an organ is infected as well as upon the severity of the disease.

Both phenomena probably occur in curly-top beets. The disintegration of chloroplasts was very distinct in leaves where they were well developed before hypertrophy began. In younger leaves containing very small chloroplasts, disintegration is less marked. In severely affected plants, hypertrophy along the veins may perhaps start so early as to inhibit the development of the chloroplasts.

Investigators report destruction of chloroplasts by living organisms (27, 62); dissolution (22); swelling (5, 6); and fragmentation (5, 6, 20, 32, 33, 46), which was the only phenomenon observed during these studies.

Vacuolation of cells, as observed in the case of virus, fungal, and bacterial diseases, has been interpreted as a sign of superactivity in the affected cells (23, 24, 25). This condition may also be induced by nonliving agents, both physical and chemical (39).

The findings of this investigation, considered in conjunction with the facts just cited, justify the conclusion that certain cells in translucent veins are in a stage of degeneration. Hypertrophy and hyperplasia, degenerative changes that, in the phloem, are somehow related to necrosis, in the mesophyll are accompanied by only partial destruction of cell contents. Clearing of the veins is a manifestation of the early stages of degeneration occurring in and along the vascular bundles. Although hypertrophy and hyperplasia may be recognized in the phloem at the stage of cleared veins, the translucency of veins seems to be explained mainly by enlargement, division, and close packing of mesophyll cells adjacent to the phloem, and by the reduction of their chlorophyll content.

Overgrowths on veins.—Veins of diseased leaves become thickened and distorted. Upon them appear numerous overgrowths of various sizes, some representing only slight irregularities on the surface, others developing into long papillae. Translucent veins, swollen veins, and wart-like protuberances are successive manifestations of one continuous process—the hypertrophy and hyperplasia of the parenchyma tissue about the phloem. At the translucent venation stage, as was mentioned above, division of cells,

followed by their radial elongation, occurs in the mesophyll adjacent to the phloem. Divisions in planes other than radial also occur during the development of a protuberance, but the radial elongation of cells is a more important cause of the extreme length of the protuberances. The long cells have but few chloroplasts and are surrounded by apparently normal cells containing a larger number of chloroplasts. The anatomical structure of healthy and of proliferated lateral veins is shown in figures 10, A, and 10, B.

Where hypertrophy and hyperplasia are not so extensive, the veins are merely thickened. In dwarfed, severely affected leaves, mesophyll other than that adjacent to bundles may be in a hypertrophied condition, resulting in abnormal thickness.

The overgrowths are always found in connection with degenerated phloem in the bundle. Either necrotic tissue or healed-over lesions may be associated with a protuberance. Townsend (65) does not make this clear in his illustration. The bundle associated with the overgrowth, as shown by him, does not differ from the healthy bundle. Abnormal xylem may be present in the degenerated phloem region (Fig. 10, B), a part of the abnormal xylem strand running parallel to the normal xylem strand in the lateral vein. No xylem occurs in the pericycle and phloem region of a healthy lateral vein.

A certain degree of hypoplasia is involved also in the formation of protuberances, no collenchyma developing below the epidermis of a protuberance, as in a healthy vein (Figs. 10, A, and 10, B). The xylem is also in a hypoplastic condition.

Consumption of cell contents and an early death do not occur in proliferations on sugar-beet leaves as they do in hyperhydric tissues. They have a certain similarity to tumors or galls induced by animal parasites, being more or less permanent structures, formed not only through hypertrophic but also through hyperplastic processes. The galls, however, show a more complex structure and frequently a more definite organization.

Proliferations, morphologically similar to the protuberances in curly-top sugar beets, may be induced in plants by mechanical and chemical means, as well as by other diseases and parasites. Schilling (51) induced overgrowths on certain plants by covering small areas on stems with paraffin. Smith (57, p. 477) obtained hyperplasias by treating plants susceptible to crown gall with chemicals said to be produced by the crown-gall organisms. According to this investigator, overgrowths may also be induced by slight freezing, mechanical irritation, over-watering in a confined atmosphere, semi-asphyxiation, etc. Although the agents that stimulate the production of hyperplasias are widely different, they produce similar

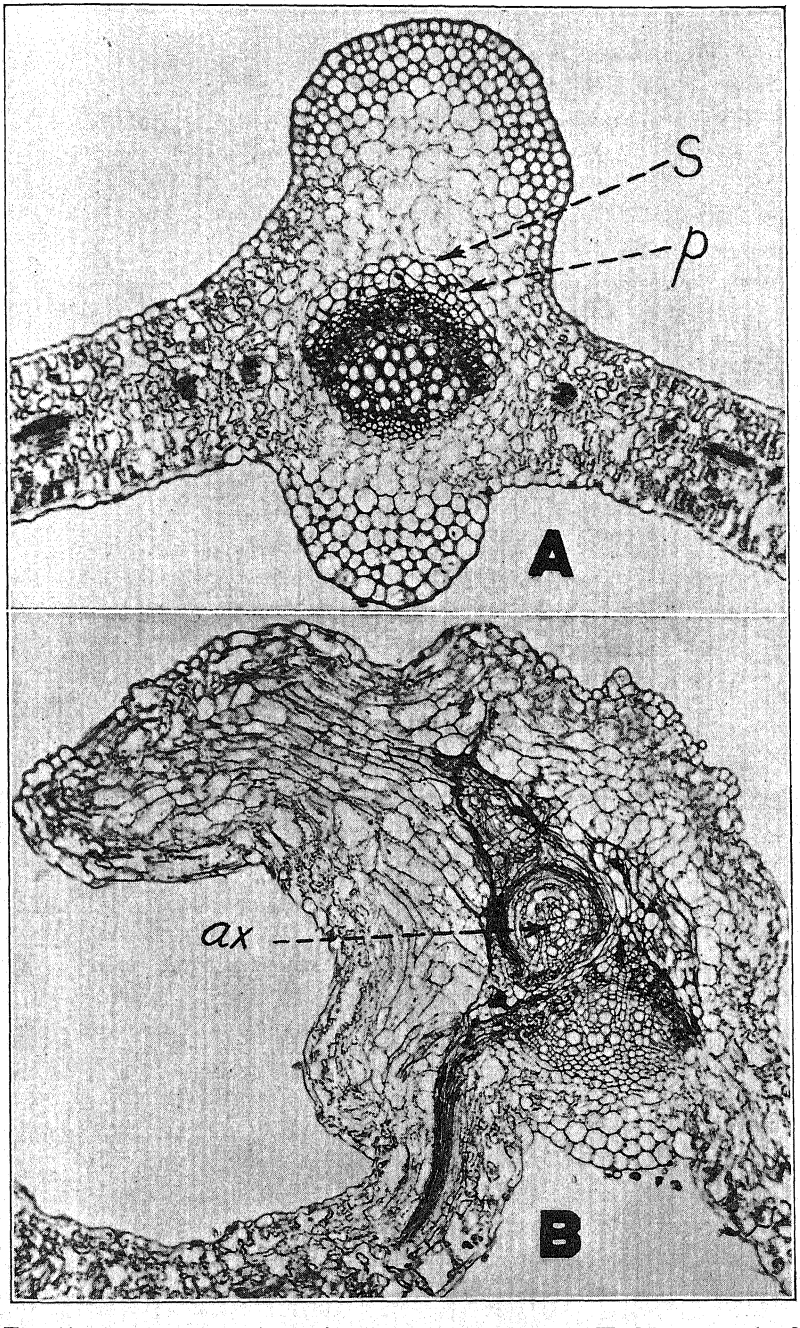


FIG. 10. Transverse sections of lateral veins. ×73. A. Healthy, *p*, pericycle; *s*, starch sheath. B. Diseased, showing proliferation and radial elongation of parenchyma cells external to bundle. Abnormal xylem (*ax*) in bundle cap region.

structures. This fact suggests that the causal factor might be the same in every case (38, 57). Possibly the stimulus to growth and division is furnished by some definite physico-chemical change that may be induced by various means. Smith's (57, p. 511) concept is that this type of change is initiated in injured cells and that all tumors begin in injuries and may be regarded as "excessive and continually modified wound-repair reactions." Gardner (29) has also found that proliferations in tomato-streak mosaic are associated with necroses.

Since the proliferations of tissues along the veins in sugar-beet leaves affected by curly top resemble those observed in other plants, suffering from other types of diseases and injuries, their development might be attributed, not to a specific action of the curly-top virus, but to some cause that is similar in the case of all such structures. Growth and cell division might be stimulated by processes involving changes in the concentration of cell constituents. An accumulation of sugars in curly-top leaves has been shown to occur and was interpreted as the result of disturbed translocation in the degenerating phloem (15). Like the hyperplasias studied by Smith (57) and Gardner (29), the overgrowths in sugar beets are associated with necrotic lesions and the stimuli that lead to formation of the hyperplastic tissue near the lesions and to the proliferations of tissues farther removed from the lesions may perhaps be similar.

DISCUSSION

The disease known as curly top induces, in affected plants, anatomical changes, which involve hypertrophy, hyperplasia, hypoplasia and necrosis. The change last mentioned is limited to the phloem and pericycle. Pathologic effects of similar nature have been observed in many other virus diseases.

The effect of virus diseases is commonly noted throughout the plant. Curly top is such a disorder, necrosis of the phloem occurring in leaves, stems, and roots. Leaves developing after infection usually show symptoms and are stunted; the root is retarded in its growth and produces numerous laterals.

The first sign of degeneration has been observed in the phloem and pericycle and is probably the primary effect of the disease. Cell proliferations in the phloem following necrosis, hypertrophy, and hyperplasia in the mesophyll, and the general stunting of the plant, may be interpreted as secondary effects. These may conceivably be responses to a disturbed nutrition caused by degeneration and necrosis of phloem. If disturbance in the phloem results in sugar accumulation in certain exterior cells, the latter may be stimulated to growth and division, thereby producing proliferations along the veins. Similar nutritional disturbances may cause cell prolifer-

ations adjacent to necrotic lesions. The stunting of the plant undoubtedly results from a deficiency in nutrition, caused by phloem degeneration.

One might suggest, then, that the primary change in the plant, phloem degeneration, is induced by the causal agent of curly top in this tissue; that the secondary changes are, at least in part, responses of the plant to internal injury and necrosis and to the effects of these upon conduction and organic nutrition. At the same time, the alternative hypothesis, that stimulation to growth and division of cells outside the phloem is a direct effect of the virus, cannot be rejected at this stage of investigation. The studies seem to indicate that among the early responses in the phloem to infection are hypertrophy and hyperplasia. Very soon this stimulation spreads to cells outside the phloem. If hypertrophy and hyperplasia in the phloem result from the presence of the virus, the stimulation of mesophyll cells adjacent to the phloem may have the same cause. It is difficult to conceive that the virus would be strictly limited to the phloem in leaves so young that all cells are still predominantly immature. The hypertrophy of cortex cells along the path of the exudate may also be interpreted in 2 ways: stimulation may be caused by increased nutrition or by the presence of the virus in the exudate.

Whether the virus stimulates cells by disturbing their nutrition, or whether it has a more specific action, remains an open question. Neither do we know whether cells proliferating near necrotic lesions in curly-top beets, as well as in other plants, are stimulated solely by nutritional disturbances, or whether specific substances (wound hormones of Haberlandt) are involved. These questions will probably not be answered until we know more about the stimuli underlying the growth and division of cells, whether they be primarily or secondarily meristematic.

Although the changes in the tissues outside the phloem may be caused, in part, by the same stimulus that leads to hypertrophy and hyperplasia in the phloem, the observations strongly indicate that the phloem and pericycle are the first tissues injured and that the effect of the virus is centered in this region. This conclusion seems significant with regard to the localization of the curly-top virus in the affected plant.

According to Küster (38, p. 383), necrosis in plants is often limited to vascular bundles, either because their elements are particularly susceptible to certain injuries, or because, in many cases, they form the channels of distribution for injurious agents.

Studies on the physiology of virus diseases indicate that some viruses probably move mainly through the phloem. Ringing experiments have shown that certain viruses travel through the bark (4, 7); and studies of the aucuba mosaic of the tomato have demonstrated that the virus does

not enter xylem (12, 13). The rates at which certain viruses have been found to travel through plant tissues (54, 63) are comparable to those reported for the translocation of materials in the phloem. Henderson Smith (58) suggested that, because the rate of spread of certain viruses is too high to be explained by movement from cell to cell, and too slow for conveyance with the water stream, this transport is evidently effected through the phloem.

The curly-top virus moves rapidly through plant tissues (54), including phloem necrosis throughout the plant. The suggestion seems justified that the causal factor of curly top is active mainly within the phloem and that disorders occurring outside of this tissue are mostly secondary—the results of inhibited conduction.

SUMMARY

Curly top induces pronounced anatomical changes in affected leaves, involving hypertrophy, hyperplasia, hypoplasia, and necrosis.

Necrosis has been observed only in the primary and secondary phloem and pericycle. It leads to a collapse of cells and formation of lesions.

Hypertrophy and hyperplasia occur in the phloem and pericycle before and after necrotic lesions are formed. Stimulation to growth and division of cells adjacent to lesions resembles wound-healing reactions. Phloem parenchyma, starch sheath, and cortical parenchyma participate in the proliferations near the lesions.

Abnormal xylem frequently differentiates in the proliferated tissue in the phloem region. Within leaves, this xylem is not connected with the normal xylem. Although phloem parenchyma and pericycle in roots and stems usually give rise to secondary cambium, which differentiates into xylem and phloem, in the leaves, such a development was found to occur only at the base of the petioles, whereas the abnormal xylem is produced in petioles, midrib, and lateral veins. The abnormal xylem differs from the secondary xylem, arising in the pericycle and phloem region, in the characteristics of its components: normal xylem has scalariform, scalariform-reticulate, and pitted vessels and pitted fibers; the abnormal xylem has spiral, scalariform, and scalariform-reticulate vessels and no fibers. The abnormal xylem shows very irregular distribution.

Hypertrophy and hyperplasia are not confined to the phloem but occur in cortical tissue also. The mesophyll cells adjacent to the phloem are stimulated to growth and division, becoming closely packed, with no intercellular spaces. The chloroplasts are few, small, and pale in the hyperplastic tissue; they are often of an irregular shape, indicating degeneration. These changes are held responsible for the symptom called clearing of veins.

In later stages hypertrophy and hyperplasia in cortical tissue lead to thickening and distortion of the veins and to a formation of protuberances. The mesophyll farther removed from the phloem may also be in a hypertrophied condition, causing thickening of leaves.

Hypoplasia is manifested mainly in the dwarfing and stunting of the plant.

It is suggested that injury and degeneration of phloem elements probably result from the presence, within the cells, of the causal agent in curly-top infection. Changes occurring in the phloem region after necrosis and those occurring outside the phloem may be interpreted as secondary responses to internal injury and to the resulting necrotic condition in the phloem. On the basis of this interpretation, a hypothesis has been advanced that the virus of curly top is active mainly in the phloem.

DIVISION OF BOTANY, COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

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CULTURE OF PHYTOPHTHORA INFESTANS

WILLARD CROSIER

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Phytophthora infestans (Mont.) de By., pathogen of the common late blight of potatoes, is often desired for experimentation and for class demonstrations in plant pathology and in mycology. It is frequently the case, however, that considerable difficulty is encountered in culturing the fungus. Ordinary culture media, such as potato-dextrose agar, oatmeal agar and cornmeal agar, which are useful in growing other species of Phytophthora, support only a feeble growth of *P. infestans*. A number of workers, including Clinton (2), Jones *et al.* (3), Rosenbaum (6), and Tucker (7), comment on the extremely meager growth of *P. infestans* on vegetable and cereal agars and on synthetic media. It is not impossible to grow the fungus on oatmeal agar but the resultant development of mycelium and sporangia necessitates the use of an unwieldly number of test tubes, Petri dishes, or flasks.

The possibility of culturing the fungus on oatmeal agar as a means of maintenance only and its transfer to potato tubers or foliage to develop large numbers of sporangia or to demonstrate the pathogen-suscept relationship has been suggested. It has been found that mycelium transferred to tubers in some cases causes a tuber rot but in the majority of cases does not. Practically all of the students who have attempted to develop a tuber rot by this method have been unsuccessful.

The best method of growing the fungus in pure culture is that of using raw aseptic tuber plugs as suggested by Jones *et al.* (3) and as improved by Löhnis (4). This method consists of surface sterilizing tubers and removing plugs of the inner tissue with a sterilized cork borer. The plugs are then placed in sterilized test tubes containing a small amount of water. This method has not been very popular since it necessitates very careful work in the preparation of the plugs, and the surface available for the formation of the aerial mycelium and the sporangia is not large enough to supply sporangia in quantity.

In a recent investigation dealing with sporangium production and germination it was necessary to develop sporangia in abundance at will and to be able to remove them for use while they were still viable. The method mentioned above was not satisfactory. It has been found much better to grow the fungus continuously on either disinfected tuber slices or on potato foliage. Transfers are made as often as necessary to maintain a thrifty clean growth. When plants of a susceptible variety are available they are inoculated with either sporangia or preferably with swarm spores. If only

a few sporangia are desired a few leaflets may be inoculated, or if a large number of sporangia are needed for extensive study or for demonstrations several plants may be thoroughly inoculated. The spores are either sprayed on with an atomizer, dropped on from a pipette or sprinkled on with a common clothes sprinkler. At an incubation temperature of 20° C. lesions appear in 3 to 5 days. The diseased leaves are then detached and placed in a moist chamber to permit sporulation. Sporangia form in abundance over the entire necrotic area and in the surrounding margin of green tissue in 8 hours or more, depending on the temperature. When it is desired to maintain the fungus for an indefinite period with a minimum of attention the plants are placed at a temperature as low as 10° C., if possible. The fungus grows slowly at this temperature, so that the plants are not killed for several weeks. When sporangia are needed the plants are placed in a moist chamber at 18 to 20° C. Under these conditions the fungus rapidly extends throughout the host plant and sporulates readily and abundantly.

When it is inconvenient or impossible to employ plants for substrata, thin slices cut from washed and surface-sterilized tubers may be utilized. Sound tubers are washed in warm water, dipped in $HgCl_2$ (1 to 1000) for 5 minutes or longer, rinsed in sterilized water and cut into slices with a sterilized scalpel. Under ordinary conditions it is necessary only to wash the tubers, dip them in 70 per cent alcohol, and flame them to insure a moderately clean and sterile surface. The slices are immediately placed in clean Petri dishes containing moist filter paper.

The fungus may be transferred to the slices by diseased tissues, aerial mycelium, sporangia or swarm spores. Either of the first two may be stabbed into the upper surface of the slice with a flamed scalpel. In case a suspension of sporangia or of swarm spores is used for the inoculum a small depression or well is made in the slice and a drop of the suspension placed therein. This permits the fungus and also the bacteria to radiate from a common focus, and the more rapidly growing fungus is enabled to advance practically free of inhibiting bacteria. The Petri dishes are then covered for at least 2 hours to maintain that high relative humidity essential for the growth of the germ tubes and for their penetration into the new substratum. Establishment of the fungus proceeds most rapidly at temperatures of 9 to 24° C., with an optimum at 19 to 22° C.

The further treatment of the slices depends on whether a new growth is desired in the briefest possible time or only after a longer period. The optimum conditions for incubation are: a temperature of 19 to 21° C. and a relative humidity of approximately 100 per cent. Under these conditions aerial mycelium appears on the slices within 2½ to 3 days, depending somewhat on the variety used for the substratum. The development of aerial mycelium and, therefore, sporangia, is retarded by a slight decrease in the

relative humidity and is entirely prevented when the latter is below 90 per cent. Subjecting the slices to low relative humidities results in decreasing the aerial mycelium and, what is more important, retards the growth of bacteria. It has been found that infected tubers or tuber slices exposed to ordinary room temperatures and humidities will not rot entirely for several weeks and that when afterwards placed in a moist chamber will give rise to sporangia in abundance. Bacterial contamination also is decreased or entirely eliminated by incubating the slices at temperatures below the optimum (19 to 21° C.) for growth of the fungus. Cultures have been held at 6° C. for as long as 30 days with no appreciable amount of contamination due to bacteria. The initiation of sporulation is considerably delayed and its continuation favored by the lower temperatures. In a number of experiments slices were placed at 6° C. within a few hours following transfer of spores and examined daily. Aerial mycelium only rarely formed within 15 days and sporangia usually appeared in about 20 days. This indicates that the fungus may be maintained with very little attention by growing it at a low temperature. At an incubation temperature of 9° C. mycelium appeared in 12 to 14 days, at 12° C. in 6 to 7 days and at 15° C. in 4 to 6 days.

A lot of Rural Russett tubers, artificially inoculated with *Phytophthora infestans,* were held from October, 1931, to July, 1932, at a temperature of 4 to 6° C. and a relative humidity of 80 to 85 per cent. Very little bacterial rot was evident at the termination of this period, although some of the tubers were nearly destroyed by Phytophthora rot. The fungus was induced to grow vigorously and to sporulate copiously simply by placing the tubers in a saturated atmosphere at 18° C. Fructifications formed in abundance in 24 hours or less. More or less similar conditions exist in ordinary storage of tubers over the winter months and similar overwintering of the fungus can be considered an actuality.

It has been shown that the production of sporangia of *Phytophthora infestans* is of common occurrence when the atmosphere is saturated with water vapor and takes place over a temperature range of 6 to 24° C. The results of several experiments show that sporulation occurs at a minimum temperature of 3° C. and at a maximum of 26° C. Vowinckel (8), working in Germany, reports essentially identical results. Only a few of the sporangia that form throughout this range of temperatures will germinate either by germ tubes or by swarm spores unless precautions be taken to maintain their viability and to supply the correct conditions for their germination. *P. infestans* in common with most species of the order Peronosporales forms aerial sporangia only when moisture is present and these sporangia remain viable and germinate only in the presence of moisture or, at least, a very high relative humidity.

Under optimum conditions the sporangia remain viable for only a few days. A high relative humidity is absolutely essential for maintenance of viability, and low temperatures prolong the period of viability. Melhus (5) observes that sporangia, held for 6 hours in a dry laboratory, are not able to germinate; while Löhnis (4) reports that no sporangia survive an hour of rapid drying. Other workers state that low humidities and high temperatures adversely affect the longevity of the sporangia, but data based on even approximately controlled factors have not been available until the last few years. Vowinckel (8) attempted to find the relative humidities necessary for sporangium formation and longevity but was not very successful because of poor control of humidity.

In a series of experiments in which the relative humidity has been controlled to within 1½ per cent it has been found that the lower the relative humidity the more rapid the loss of viability. Lots of sporangia that gave 80 per cent indirect germination at 13° C., as soon as mature, have been held at a temperature of 20° C. and various relative humidities. Sporangia exposed to 99 per cent relative humidity for an hour gave 72 per cent indirect germination; those held at 90 per cent for an hour gave 45 per cent germination; those at 50 per cent gave 11 per cent germination; and those held at 25 per cent gave only 5 per cent indirect germination. Similar lots of sporangia have been exposed to temperatures of 25 and 30° C. and various relative humidities. In these cases the loss of viability was even more pronounced. As a comparison, sporangia exposed to 25° C. and 90 per cent relative humidity for an hour gave 40 per cent indirect germination, whereas those held at a similar relative humidity and at a temperature of 30° C. gave only 32 per cent. indirect germination. Reduced humidities and high temperatures affect the ability of the sporangia to germinate directly, although to a lesser degree.

The experiments of Melhus (5) demonstrate that a greater percentage of sporangia will germinate by swarm spores at 13° C. than at any other temperature to which he exposed them. The optimum temperature for germ-tube formation is reported to be 24° C. It has been found in similar experiments that sporangia of *Phytophthora infestans,* regardless of their age or the conditions under which they have been stored, germinate most rapidly and abundantly at 12 to 13° C. by swarm spores and at 24° C. by germ tubes. The range of indirect germination extends from 1° C. or lower to about 25° C. and that of direct germination from 6° C. to nearly 30° C. The curves in figure 1, A, show the relation of temperatures from 1½ to 30° C. to both indirect and direct germination of variously treated sporangia. A considerably greater percentage of sporangia germinate by swarm spores (curves IM, IO, IE) than by germ-tube formation (curves M, O) at temperatures of 1½ to 18° C., whereas the opposite is true at temperatures

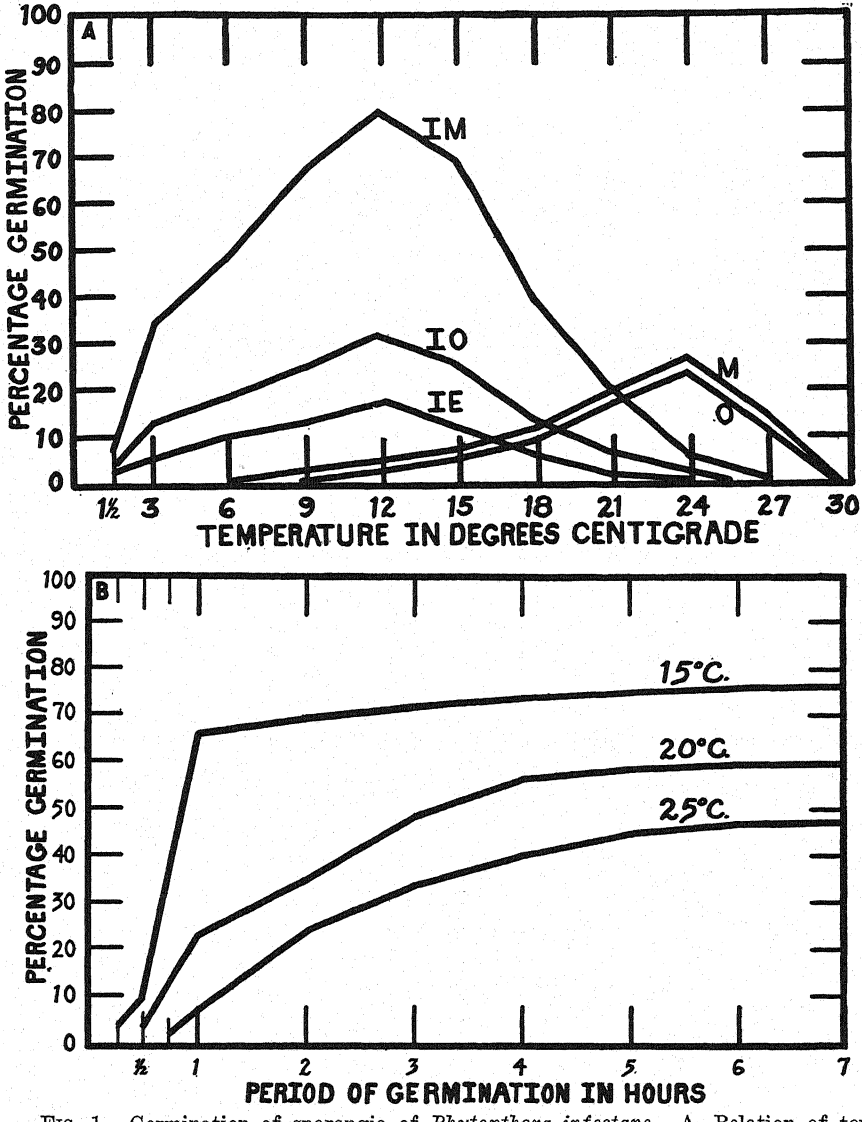


FIG. 1. Germination of sporangia of *Phytopthora infestans*. A. Relation of temperture to indirect germination (IM, IO, and IE) and to direct germination (M and O). The curves are based on the actual percentages of sporangia that had germinated after exposure for 50 hours to the indicated temperatures. B. Rapidity of indirect germination at 13° C. Sporangia were removed from cultures that had been incubated at 15, 20, and 25° C.

of 21 to 30° C. The relation of age and storage conditions of the sporangia to indirect germination is shown by curves IM, IO, IE. Old sporangia, curve IO, and sporangia that have been exposed a few hours to a dry atmosphere, curve IE, germinate less rapidly and also less abundantly than freshly matured sporangia, curve IM. It is evident from curves M (mature sporangia) and O (old sporangia) that the same relation of age to percentage of germination exists in direct germination of the sporangia, although it is by no means so marked as in indirect germination.

The rapidity of indirect germination of sporangia immersed in water and exposed to the optimum temperature of 13° C. is shown in figure 1, B. The influence of the temperature under which the sporangia have developed and have been stored is shown by the three curves. The most rapid and abundant germination occurred in these lots of sporangia that had formed on slices and leaves incubated at 15° C. Sporangia of the same age, formed and held at 20 or 25° C. until placed under conditions requisite for germination, germinated less rapidly and less abundantly. This indicates, as field observations have confirmed, that, even though the relative humidity approach 100 per cent, sporangia that are exposed to temperatures of 25° C. or higher for a few hours germinate very poorly, if at all, whereas those subjected to a temperature not exceeding 15° C. remain viable for several hours. No germination of sporangia has ever been observed unless water be present in the liquid state. The existence of a high relative humidity of 98 to 100 per cent does not suffice; the sporangia must be in contact with a film of water. Ordinarily, a slightly better germination occurs in a shallow than in a deep receptacle, and the results of many experiments show the presence of vaseline, bentonite clay, or finely dispersed infusorial earth increases the percentage of indirect germination.

If one wishes to demonstrate the phenomenon of swarming, the swarmspore suspension should be held at a temperature as near as possible to 3° C. The duration of motility decreases from 22 hours at 3° C. to 30 minutes at 24° C. A culture of swarm spores thus can be held for several hours in a motile condition simply by placing them at a low temperature. It also has been found advantageous in inoculation experiments to place the swarmspore suspension at 3° C. if plants are not ready for inoculation as soon as the swarm spores have formed. The swarm spores, after coming to rest, germinate readily at 12 to 24° C. and less readily at 3 to 9° C. The optimum temperature in relation to percentage of swarm spores that germinate is 15° C., while the germ tubes elongate most rapidly at 21° C. and die quickly at 26° C. or above. In inoculation work the foliage of the plants should be kept moist for at least 1½ hours if the temperature is around 20 to 25° C., 2 hours if 15° C., and 2½ to 3 hours if 10° C. The

percentage of penetration of germ tubes, however, continues to increase for as long as 8 hours at 10 to 25° C. After this period there is no particular advantage in keeping the plants in a moist atmosphere. Numerous inoculation experiments have been made using a swarm-spore suspension containing 10 spores to a drop. At 10° C. only 4 per cent of the inoculations resulted in infections when the plants remained moist for 3 hours, but 85 to 90 per cent of the inoculations were successful when the plants were kept in the moist chamber for 8 hours. Comparable percentages for 15° C. are 9 and 88, and for 21° C. are 13 and 91.

As previously mentioned, the optimum temperature for direct germination of the sporangia (conidia) is 24° C. The process of germ-tube formation is, compared to formation of swarm spores, relatively slow and rarely more than 20 per cent of the sporangia germinate directly. In only one case have germ tubes been observed until the sporangia had been at the optimum temperature of 24° C. for over 4 hours. Ordinarily some germination occurs in 8 hours at 18 to 26° C., although some lots of sporangia held at 24° C. have not started to form germ tubes until the third day. It is possible, as Blackwell and Waterhouse (1) mention, that maturation of the sporangia is a factor in the percentage and rapidity of direct germination. From the facts that more sporangia germinate indirectly than directly, that one sporangium may form 8 to 20 swarm spores but only one germ tube, and that the process of liberation of swarm spores and their subsequent germination consume considerably less time than the formation of germ tubes directly from the sporangia, it is to be expected, as many experiments have shown, that *Phytophthora infestans* spreads rapidly in the field when the temperature favors indirect germination of the sporangia but very slowly when the temperature favors direct germination.

SUMMARY

Suggestions are given for culturing *Phytophthora infestans* in pure culture and on potato tubers and foliage.

Bacterial contamination is eliminated by incubating the tuber cultures at low temperatures or at low relative humidities.

Sporulation occurs over a temperature range of 3 to 26° C. A saturated atmosphere is essential for sporangium formation.

A high relative humidity is necessary to preserve viability of the sporangia. The period of viability also is increased by holding the sporangia at low temperatures.

The optimum temperature for indirect germination of the sporangia is 12 to 13° C., and for direct germination is 24° C.

The sporangia of *P. infestans* germinate only in the presence of water. Bentonite clay and certain other substances increase the percentage of indirect germination.

Swarm spores of *P. infestans* may be maintained in a motile condition by holding the spore suspension at 3° C.

A small percentage of the swarm-spore germ tubes penetrate the host tissue in 1½ to 3 hours. Penetration proceeds most rapidly at 20 to 25° C.

The results of many experiments tend to prove that *Phytophthora infestans* spreads most rapidly in the field when the temperature favors indirect germination of the sporangia.

CORNELL UNIVERSITY,
ITHACA, N. Y.

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ASCOSPORE DISCHARGE IN OPHIOBOLUS GRAMINIS, AND ITS PROBABLE RELATION TO THE DEVELOPMENT OF WHITEHEADS IN WHEAT

GEOFFREY SAMUEL AND S. D. GARRETT

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The epidemiology of the take-all disease of wheat, caused by the fungus *Ophiobolus graminis* Sacc., is comparatively little understood as yet. There have been two main suggestions as to the method of dissemination of the fungus in the field: (1) that "ascospores constitute the inoculum for the vast majority of infections" (Kirby (3)) and (2) that mycelium, carried by the wind with soil particles and in pieces of tissue from the bases of infected cereals and grasses, forms an important additional method of spread (McAlpine *et al.* (4)).

A number of workers have studied ascospore discharge, and most agree that when ripe perithecia are placed in water the asci are ejected singly into the water, where they quickly burst, liberating the spores. Waters (5), however, figures asci and ascospores being extruded in a tendril through the ostiole.

Kirby (3) stated "it is clear that the behavior of the ascospores at the time of their discharge restricts the agents of dissemination almost completely to rain." He also considered that the delicate nature of the ascospores rendered them unsuitable for dissemination by wind, and he determined that spores from a drop of water, allowed to dry on a glass slide, lost their power of germination within half an hour.

The two statements of Kirby, quoted above, seem entirely inadequate to explain the known field occurrence of take-all. If ascospores are the main source of infection and if they can be disseminated only by rain washing them about in the soil, the appearance of take-all in the whitehead stage throughout 100-acre fields of wheat sown on fallow, as frequently occurs in Australia in seasons favorable to the disease, would be impossible to explain.

We have recently made the observation that during periods of rain ascospores may be ejected from the perithecia into the air at the rate of several hundred per minute from a single diseased wheat culm, in much the same manner that the ascospores of *Venturia inaequalis* are ejected during rainy periods in spring from dead apple leaves on the ground. In addition to this, fields badly infected with whiteheads were found to have a number of small patches in them in which the plants had become diseased in the early stages in the typical take-all manner and on which perithecia were present. It is considered that these two facts, combined, throw new light on the epidemiology of take-all.

INCIDENCE OF TAKE-ALL IN SOUTH AUSTRALIA

The incidence of take-all in the wheat fields of South Australia may be described briefly as follows: Crops sown on new land just cleared from Eucalyptus scrub are usually entirely free from the disease. It is the practice to take 2 or 3 wheat crops in succession off such land, and in the second and third years isolated plants or small patches affected with take-all can often be found and occasionally there is a quite serious infection. After this period, depending on the weather, the growing of further wheat crops may become a gamble unless oats or bare fallow is brought into the rotation. Take-all definitely becomes the limiting factor to the frequency of cropping with wheat. As time goes on and farms are brought into full cultivation, with the adoption of good crop rotations, methods for better consolidation of the soil, improved manurial practices, etc., take-all gradually becomes comparatively rare unless induced by special circumstances. The older wheat-growing districts are, therefore, frequently spoken of as having "passed through the take-all phase," and the disease is no longer feared.

EPIDEMICS

Epidemics of take-all appear to result in this country from the coincidence of 3 main factors: (1) lack of consolidation of the soil, (2) the presence of some inoculum in the soil, and (3) last, but not least, the occurrence of showery weather lasting almost until the crop is in head.

By far the greatest loss from take-all is due to the development of the whitehead stage of the disease just before the crop is ripening. Splendid crops, 4 ft. or more in height, in which only a comparatively few take-all patches were evident before flowering, may suddenly develop whiteheads after flowering, so that more than 80 per cent of the grain is lost and nothing can be done but reap the crop for a poor-quality hay, or burn it. Widespread losses of this type occur only in years when the ripening weather is showery and only on soils not well consolidated. The serious nature of the disease in seasons favorable to it is evident from the fact that in the State of South Australia, alone, take-all took in 1932 a toll of several million bushels of wheat.

The 3 factors mentioned above as determining the occurrence of serious epidemics of take-all may be discussed in somewhat further detail as follows:

(1) *Consolidation of the Soil.* Most of the affected crops are on land of a light, sandy texture. Moreover, instances frequently occur of a crop on light land remaining healthy only in parts where the soil has been well compacted. The following instance, given by a farmer on Eyre Peninsula, is fairly typical: "The paddock fallowed was 170 acres and sheep had access to it the whole season, a gate being left open between the fallow and

a grass paddock containing water. About 50 acres in the vicinity of the gate, and some 10 acres on a rise where the sheep made a camp, were so solidly compacted by the sheep that the wheat on these portions should yield 24 bushels to the acre. The crop is healthy, dense, and of good even colour, while on all other parts of the fallow take-all is very severe."

In the effort to get maximum consolidation of the lighter soils of the mallee wheat-growing areas, the best farmers plow only about 2 in. deep and keep their fallow lands clean either with sheep or with tined cultivators working to a shallow depth (1).

Take-all, however, can occur also on heavier land. In such cases it usually has been induced either by too deep plowing or by plowing in pasture grasses and cropping the following year, especially when seasonal conditions have not allowed a proper compacting of the soil. The deepest-plowed plot (12 in.) in the depth-of-plowing tests at Roseworthy Agricultural College is always the plot most severely affected by take-all.

It cannot yet be said whether the greater severity of take-all on soils that are not well consolidated is due to the wheat plants being less resistant to the disease under such conditions, or to there being more favorable conditions for the activity of the fungus in loose soils, or to both of these factors acting together.

(2) *Inoculum in the Soil.* Very little is known about the distribution and concentration of take-all inoculum in the soil. Since, in a favorable year for take-all, a field in which the soil is poorly consolidated will practically always suffer severely from the disease, the opinion seems to be current in Australia that the take-all fungus is present almost everywhere in the soil, awaiting only favorable conditions to become an active parasite. But, there are several facts that would be very difficult to explain on this hypothesis. Why should a few take-all patches show up early in the season and kill the plants in the seedling stage, whereas by far the greatest loss does not appear until after flowering? The plants that succumb to whiteheads could scarcely have been growing in contact with *Ophiobolus graminis* since seeding time, and yet no method of distribution of the fungus over large areas during the growing period of the crop has yet been suggested.

The question of the amount of inoculum in the soil will be discussed in greater detail by one of us at a later date. No doubt there are circumstances when take-all inoculum might be fairly generally distributed in a soil at seeding time. This might occur, for instance, if a grass paddock in which a considerable amount of barley grass was infected with take-all was plowed late, especially if the plowing was followed by dry weather. But, the suggestion made here is that in most cases the soil of the fallows is largely free from inoculum and that the positions where inoculum exists at seeding time are the comparatively few places where take-all patches

develop in the crop in the seedling stage. Whether these "primary patches" are due to ascospore infections or to pieces of infected stubble, remaining in the fallow or being blown onto it in summer, will not be further considered here.

(3) *Weather Previous to Ripening.* It has been stated above that serious epidemics of take-all occur only in years when showery weather lasts almost until the time of ripening of the crop. Conversely, when the spring is fine and dry, comparatively little take-all in the whitehead stage can be found.

The relation between the amount of spring rain and take-all may be seen in table 1. The explanation now proposed for this relation is that ascospores, maturing in perithecia on the dead plants in the primary take-all patches, are dispersed over the field in wet spring weather, giving rise to a scattered secondary infection, manifested in the development of whiteheads.

There are no doubt other factors than spring rainfall of which account must be taken. For instance, take-all was much more severe in 1932 than in 1930, although the amount of spring rain was actually somewhat less. A possible explanation may be advanced as follows: The late rains in 1930 were no doubt favorable to infection of grass paddocks, as well as crops, and many of these grass paddocks were plowed up for fallowing about August, 1931. The subsequent dry spring and summer would have allowed more of the fungus to survive as viable initial inoculum for the 1932 patches. The rains in 1932 came early and led to earlier sowing of wheat; this may have given, by spring, an opportunity for the development of more perithecia than usual. When the spring of 1932 also happened to be wet everything was apparently favorable for the severe epidemic of take-all that actually occurred.

TABLE 1.—*The approximate severity of take-all on poorly consolidated soils in the mallee wheat-growing districts of South Australia since 1921, together with the September–October rainfall at Adelaide*

Year	Sept.–Oct. rain	Take-all	Year	Sept.–Oct. rain	Take-all
1921	4.9	Severe	1927	1.4	Light
1922	3.3	[a]	1928	4.2	[a]
1923	8.1	Severe	1929	3.2	Light
1924	5.5	Severe	1930	5.4	Fairly severe
1925	3.3	[a]	1931	3.3	Light
1926	4.7	[a]	1932	4.3	Severe

[a] No definite reports available, but the disease could have been only light to moderate or it would have been definitely noted.

SPORE DISSEMINATION

The method of ascospore discharge from the perithecia was discovered in the laboratory. Specimens of diseased wheat plants had been obtained from take-all patches in a crop of wheat some 5 ft. high, which was rapidly going off with whiteheads. There were only a few of these early diseased patches, but the plants in them had died when only about a foot high, and a number of perithecia were present on the bases of the culms. Small pieces of leaf sheath, containing perithecia, were cut out, laid on glass slides, and allowed to soak up drops of water until fully moistened. Clean cover glasses were suspended about a millimeter above them, resting on 2 matches. The preparations were kept under observation through a binocular microscope.

Spore ejection commenced in most cases some 10 to 30 minutes after moistening. Directly above the protruding neck of each ripe perithecium the spores were observed hitting the under surface of the cover glass in groups of 8 as each ascus was extruded and burst. The 8 spores hit the glass simultaneously and adhered to it. Single perithecia ejected asci at a rate of from 2 to 10 per minute, when in good condition, the rate gradually slackening and ceasing entirely after an hour or 2. A piece of tissue about $\frac{1}{8}$-inch square with 6 perithecia on it ejected spores at the rate of considerably more than 100 per minute for over an hour. Fresh cover slips were put on every minute and were then placed in Petri dishes for the longevity tests described below.

The same type of spore ejection was proved to occur in the field when perithecia-bearing culms were moistened with rain water. By holding a sticky glass plate behind them and gently blowing, it was also shown that the ascospores could be carried away in air currents as they were ejected.

Since the asci in the perithecia of *Ophiobolus graminis* are not all ripe at once, it is probable that ascospore discharge takes place from the same perithecia on many successive occasions during a rainy spring. Spore ejection has been obtained from small pieces of tissue in the laboratory on several successive days.

Occasionally, perhaps when the perithecia are not fully moist, the forcible projection of the ascospores as the ascus comes to the mouth of the perithecium fails to take place. There is then a gradual accumulation of asci at the ostiole, but nothing so marked as some of the coiled tendrils figured by Waters (5) has been seen.

LONGEVITY OF THE SPORES

The longevity of the spores was tested by germinating them in bean decoction at 24° C. at intervals of 12 hours following their ejection from perithecia to sterile cover slips. In each experiment all of the spores used

came from the same group of discharging perithecia. The cover slips, each with some 50 to 100 spores, were divided between 4 sterile Petri dishes. Two were kept at a temperature of 12° C. and 2 at a temperature of 24° C. Of the 2 at each temperature 1 was dry, and the other had a piece of moist filter paper inside the lid. The results of 1 series of germination tests are given in table 2. There was some variation between different batches of material, but table 2 gives results typical of good material.

TABLE 2.—*Results of germination tests at 12-hour or daily intervals on ascospores of Ophiobolus graminis. The spores were ejected onto sterile cover slips and were kept at 2 different temperatures and 2 different humidities*

Cover slips kept in incubator at	Time after ejection									
	0	12	24	36	48	60	72 hours	4	5	6 days
24° C. dry	+	+	+	+	+	+	+	−	−	
moist	+	+	+	+	+	+	+	+	+	+
12° C. dry	+	+	+	+	+	+	+	+	−	−
moist[a]	+	+	+	+	+	+	+	+	+	+

[a] Drops of moisture condensed on the cover glasses in the moist dishes at 12° C. after about 2 days, and a few of the spores germinated; these were excluded from the count when the germination test was done.

The average air temperature in the field during rainy periods in spring would not be much above 12° C.; and, since the humidity would be high, the spores probably would be viable for several days under field conditions. This would give ample opportunity for wind dissemination and for infection in a rain-sodden soil.

DISCUSSION

A number of points about the occurrence of take-all, hitherto very difficult to explain, become much easier to understand if aerial dissemination of ascospores takes place. There is not only the occurrence of early and late infection but the fact that the former is always in patches and the latter usually scattered; the relation between spring rains and the development of serious epidemics of whiteheads; the abundant infection that sometimes occurs on fallowed paddocks; the appreciable infection occasionally recorded on new land, etc.

With regard to the longevity of the spores in its relation to aerial dissemination, our method of collecting naturally ejected ascospores by suspending cover glasses close to the necks of perithecia is a much preferable way of obtaining material for longevity tests than Kirby's method of allowing spores in a drop of water to dry. A longevity of several days, for dry spores, seems a much more reasonable result than Kirby's half-hour, and would give ample opportunity for the dissemination of the fungus over considerable distances in rainy weather.

Air-filtration experiments in the field, such as those carried out by Keitt and Jones (2) for *Venturia inaequalis,* would probably lead to similar results for the take-all fungus. That is, in fine weather few spores or none are likely to be found in the air, but soon after rain falls the spore concentration probably rises considerably. In the case of take-all, however, instead of the spores infecting the aerial parts of plants, as in apple scab, they are with little doubt soon carried down in the rain over the large areas of wheat fields and infect through the roots. Further studies of root infection by ascospores will, therefore, be of much interest.

The explanation here advanced for the development of epidemics of the whitehead stage of take-all certainly does not present a complete picture of the epidemiology of the disease. In the epidemic season of 1932, in the same district in which could be found instances of severe crop losses most readily explainable on the above hypothesis, there were also to be found cases where primary take-all patches had appeared in crops and no secondary spread had occurred. This may have been due, however, to the fact that perithecia did not mature early enough on the different varieties of wheat involved, or to a difference in soil character, which rendered the wheat plants nonsusceptible at the time of ascospore discharge, or most probably to soil conditions unfavorable to infection by ascospores. Cases also were seen of a moderate (perhaps 5%) scattered whitehead infection through crops that could not be definitely traced to any particular primary patches. Diseased barley grass, bearing perithecia, was, however, found in the vicinity, and, although the infections did not seem to be markedly more numerous near the barley grass, this offers a possible explanation. There is no doubt that the field occurrence of take-all is influenced by many interacting factors (soil, climatic, and biological) and that much more information is needed concerning all of them.

Finally, with regard to the bearing of these facts on practice, some fresh problems are raised. Would the digging under of the early take-all patches be an effective control measure against the extensive whitehead infection that develops on certain soils in wet springs? At present all the emphasis is laid on elimination of the source of infection (apparently considered as extensively distributed in the soil) by means of crop rotation, combined with thorough consolidation of the soil. Both these methods are of the greatest practical value, and it is desirable that primary emphasis should continue to be laid on them. But, in a favorable season for take-all, there are often heavy losses from the disease on land that has been reasonably well-fallowed. And there are also light sandy soils in the mallee districts of Australia that cannot yet be properly consolidated by any treatment. Under these circumstances it would appear well worth while to test the third possibility of control, namely, destroying the plants in the primary

take-all patches to prevent the formation of perithecia on the dead plants, and thus eliminate what now appears to be the most likely source of secondary infection. This method, of course, would be impracticable where primary infection was widespread.

There is one fact that might render such action much less efficacious than would appear at first sight and that is that much of the barley grass, naturally present along the fences and in pasture paddocks, is often infected with take-all. It is possible that these plants may provide a source of ascospores for wind dissemination. At present the evidence would suggest that if they do so it is only to a very limited degree compared with the wheat plants in the primary take-all patches in the crop; but an answer to these and other problems will have to await further work in the field.

SUMMARY

It is shown that moistened perithecia of *Ophiobolus graminis* eject ascospores into the air. The rate of discharge may attain several hundred spores per minute from a single diseased wheat culm. The ascospores may be carried by the wind, and their longevity is in the neighborhood of from 3 to 4 days under dry conditions, and as much as a week under moist conditions, at a temperature of 12° C.

The greatest loss from take-all in South Australia occurs in the whitehead stage and in seasons when showery weather lasts almost until the crop is in head. In crops rapidly going off with a scattered whitehead infection, early diseased take-all patches were always found in which the plants had died before flowering and on which perithecia were present. Taken in conjunction with the method of ascospore discharge described above, this suggests that epidemics of whiteheads may result from wind-disseminated ascospores.

WAITE AGRICULTURAL RESEARCH INSTITUTE,
UNIVERSITY OF ADELAIDE,
SOUTH AUSTRALIA.

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WEAK BORDEAUX SPRAY IN THE CONTROL OF FIRE BLIGHT OF APPLE[1]

MONROE McCOWN[2]

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Spraying tests for the control of blossom blight of apples and pears, caused by *Bacillus amylovorus* (Burrill) Trevisan, have been conducted in Indiana orchards for the past 6 seasons. The results of earlier tests,[3, 4] showed practical control of the disease by the application of a weak Bordeaux (1–3–50) spray when the blossoms were entering the full-bloom stage. It is the purpose of this paper to present further evidence of the value of Bordeaux in fire-blight control obtained through tests conducted during the past 3 years.

THE EFFECT OF A FULL-BLOOM BORDEAUX SPRAY ON BLOSSOM INFECTION IN INOCULATED GRIMES CLUSTERS

On May 2, 1931, 400 clusters of blossoms on a Grimes apple tree in full bloom in the University orchard at La Fayette were sprayed once with Bordeaux 1–3–50 (1 lb. $CuSO_4$, 3 lbs. hydrated lime, 50 gals. water). The spray was applied by means of a small atomizer. All unopened blossoms were either opened and sprayed or removed, and the wounds thoroughly covered with the Bordeaux spray, to prevent subsequent opening of unprotected blossoms between the time of spraying and the later inoculations. Immediately following the application of the Bordeaux, 100 of the sprayed clusters were inoculated by means of an atomizer containing a cloudy suspension of the fire-blight bacteria in distilled water. Like numbers were inoculated in the same manner each day during the 3 succeeding days or approximately 24, 48, and 72 hours after spraying. Each day 50 unsprayed blossom clusters were inoculated as checks. The inoculations were made between 10 : 00 a. m. and 3 : 00 p. m. each day. The maximum temperatures recorded by the United States Weather Bureau for May 2, 3, 4, and 5 were 68, 65, 76, and 77 degrees, respectively. No precipitation occurred during

[1] Contribution from the Division of Horticulture, Purdue University Agricultural Extension Service, La Fayette, Indiana.

[2] The writer is indebted to Dr. Max W. Gardner of the Division of Plant Pathology, University of California, Berkeley, California, former Chief in Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana, for valuable advice and suggestions during the progress of the work.

[3] McCown, Monroe. Spraying for the control of fire blight in the apple. Trans. Ind. Hort. Soc. 67: 129–133. 1928.

[4] McCown, Monroe. Bordeaux spray in the control of fire blight of apple. Phytopath. 19: 285–293. 1929.

this period, but some rain fell each day from May 6 to May 13 inclusive, totaling 1.15 inches.

The test was repeated in 1932, the technique being the same as that employed in 1932, except that the spraying was done with a small hand sprayer of one quart capacity. All of the blossom clusters on several large branches in two Grimes trees in the University orchard were sprayed May 5, when the clusters were just opening into full bloom. All clusters on one branch were inoculated immediately after spraying by means of an atomizer containing a cloudy suspension of the fire-blight bacteria in distilled water. The clusters on other branches were similarly inoculated 24 and 48 hours after they were sprayed. Each day the clusters on unsprayed branches were inoculated as checks. The inoculations were made between 10:00 a. m. and 3 p. m. and the maximum temperatures reported for May 5, 6 and 7 were 82, 82, and 77 degrees, respectively. A precipitation of 0.18 inch was recorded on May 4. There was no rainfall May 5 or 6, but 0.82, 0.51, and 0.33 inches of rain fell May 7, 8, and 9 respectively, followed by very light precipitation on May 10, 11, 12, 14, 15, 16, and 17.

During both seasons, records were taken at frequent intervals in order to differentiate between two types of infection: primary, in which the bacteria had entered presumably through the nectary; and secondary, in which the bacteria had entered the base of the pedicel from the spur.

The results, recorded in table 1, show that the Bordeaux was very effective in the prevention of fire-blight infection in blossoms inoculated, both immediately after spraying and 24 hours later. In 1931, while all the check clusters were infected, only 48 per cent of the sprayed clusters inoculated immediately after spraying and 50 per cent of those inoculated 24 hours later blighted. Although 57 per cent of the untreated clusters, inoculated May 5, 1932, were infected only 10 per cent of the sprayed clusters, inoculated on the same day immediately after spraying, blighted. Since less than 5 per cent of either sprayed or check clusters inoculated at the 48- and 72-hour periods in 1931 and 24- and 48-hour periods in 1932 blighted, no records of infection in these clusters were kept. The control obtained in 1931 and 1932, reported in table 1, corroborates the results of the inoculation tests in 1927.

The poorer control obtained in 1927 in clusters inoculated 24 and 48 hours after spraying was, at that time, attributed to the fact that there was an average of one unopened blossom in each cluster at the time the Bordeaux was applied. The results obtained in 1931, *i.e.*, equal control in clusters inoculated immediately after spraying and 24 hours later, following the elimination of this factor, tend to prove this suspicion correct.

A comparison of the average number of primary infections per cluster shows an even greater germicidal action by the Bordeaux than is reflected

TABLE 1.—*Inoculation tests. Effect of Bordeaux mixture 1–3–50 applied as a full-bloom spray upon fire-blight infection in inoculated Grimes apple blossoms*

Year	Time of inoculation	Spray treatment	Number of clusters inoculated	Average no. of primary infections per cluster	Percentage of clusters infected
1931	May 2, immediately after spraying	Bordeaux 1–3–50	93	0.73	48
		none (check)	50	3.52	100
	May 3, 24 hours after spraying	Bordeaux 1–3–50	79	0.78	50
		none (check)	50	2.88	100
1932	May 5, immediately after spraying	Bordeaux 1–3–50	193	0.15	10
		none (check)	159	1.15	57

in the comparison of blighted clusters. In 1927 there was an average of 0.08, 1.08, and 1.25 primary infections per cluster respectively in the sprayed clusters inoculated immediately after spraying, 24 and 48 hours later. The check clusters for these same days yielded 1.46, 2.24, and 1.88 primary infections per cluster. Records for 1931 show an average of 0.73 and 0.78 primary infections per cluster in the sprayed clusters and 3.52 and 2.88 primary infections per cluster in the check clusters inoculated May 2 and 3. In 1932, there was an average of only 0.15 primary infections per cluster in the sprayed clusters and 1.15 in the nontreated clusters.

THE EFFECT OF AN EARLY FULL-BLOOM BORDEAUX SPRAY UPON NATURAL BLOSSOM INFECTION IN JONATHAN APPLE CLUSTERS

A block of Jonathan apple trees in the orchard of the Simpson Orchard Company,[5] near Vincennes, Indiana, was sprayed with Bordeaux 1–3–50 from one side when opening into full-bloom in 1930. A little more than one-half of each tree was thoroughly covered with the spray, and since the application was made with high pressure to well pruned trees of medium size, some of the spray material was forced through the trees and may have provided partial protection to some clusters considered as unsprayed. A row of ten representative trees was selected for records. Unsprayed and treated branches, totaling approximately the same area, on each tree were then selected and the number of infected clusters was counted on each.

[5] The writer wishes to acknowledge the cooperation of the Simpson Orchard Company for the application of the spray; and of Leslie Pierce of the U. S. Bureau of Plant Industry, Vincennes, who assisted in obtaining the data on this test.

The total area represented by the branches selected for records of each treatment approximated one-fifth of the total area of each tree.

The results show that the weak Bordeaux spray was very effective in the control of the disease on these 10 trees. While there was an average of 119 infected clusters on the untreated branches in each tree, the sprayed branches yielded an average of only 39 blighted clusters, resulting in a reduction of 67 per cent in the number of infected clusters as a result of the spray.

These results are in line with the control obtained in 1928 in Jonathan apples and Seckel pears following full-bloom applications of 1–3–50 Bordeaux. The number of infected clusters was reduced 79 and 52 per cent in Jonathan apple and 98 per cent in Seckel pear by the Bordeaux treatment in 1928.

The results obtained in these field tests and in laboratory studies previously reported,[6] in which Bordeaux apparently killed the bacteria, indicate that the Bordeaux spray provides protection to the blossoms through its germicidal action upon the bacteria after they are deposited in the blossom or upon other tissues protected by the spray.

No russetting of the fruit or reduction in the set of fruit has been observed in any of the above tests. In addition, the writer has observed the fruit in various commercial orchards in Indiana which had received a full-bloom application of Bordeaux 1–3–50 and in no case has noticeable injury resulted from the weak Bordeaux spray. No arsenate of lead has been added to the full-bloom sprays used in these tests.

The fact that an early full-bloom spray of Bordeaux 1–3–50 has given practical control of blossom infection by fire blight without apparent injury to either fruit or foliage and may, in some seasons, also prove valuable in the control of apple scab,[7, 8] warrants the use of this spray on blight-susceptible varieties in Indiana orchards.

SUMMARY

Bordeaux 1–3–50 applied early in the full-bloom period reduced fire-blight infection in Grimes blossom clusters inoculated immediately after spraying and 24 hours after treatment. In 1931, while all the inoculated check clusters became infected, only 48 per cent of the sprayed clusters inoculated immediately after spraying and 50 per cent of those inoculated

[6] *Loc. cit.* See footnote 4.

[7] Burkholder, C. L. Dusting *vs.* spraying of apples, 1927–1931. Agr. Exp. Sta. Bul. 356. 1931.

[8] Dutton, W. C. Spraying materials and the control of apple scab. Michigan Agr. Exp. Sta. Spec. Bul. 203. 1930.

24 hours after treatment blighted. In 1932, while 57 per cent of the untreated check clusters blighted, only 10 per cent of the sprayed clusters developed infection. Natural infection in Jonathan clusters in 1930 was reduced 67 per cent by one spray of Bordeaux 1–3–50 applied as the trees were entering the full-bloom period. No apparent injury to fruit or foliage has occurred in these tests following the use of a weak Bordeaux. No reduction in set of fruit has been observed.

PURDUE UNIVERSITY AGRICULTURAL EXTENSION,
LAFAYETTE, INDIANA.

OBSERVATIONS AND NOTES ON CITRUS DISEASES IN MINAS GERAES, BRAZIL

ALBERT S. MÜLLER

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The citrus industry of Minas Geraes, Brazil, is still in its infancy commercially. In most cases the older farm house plantings are closely spaced and usually show very poor growth conditions, probably because of lack of care. Large commercial orchards are as yet nonexistent in this Province. A considerable amount of experimental plantings of various citrus varieties have been started at the Agricultural College (Escola Superior de Agricultura) opened in 1927. These experimental trees are, therefore, not very old.

TRUNK AND ROOT DISEASES

In many closely spaced farmyard plantings of sweet orange, a gum disease resembling *mal di gomma* is prevalent. This is probably the most important trunk and root disease. This same malady became so serious in a row of 5-year-old seedling grapefruit trees that they had to be removed.

At the Agricultural College, citrus seedlings have been seriously affected with a similar gum disease at the base. Two blocks of Rangpur lime, one of 600 one-year-old seedlings, and the other of 1,000, each showed infections of 31 per cent and 19 per cent, respectively. In 1931 and 1932 5 different stocks, 300 seedlings each, were affected as follows:

Stock	Percentage affected with gum disease	
	1931	1932
Rough lemon	29	23
Rangpur lime	26	19
Sweet orange	26	19
Shaddock	17	11
Sour orange	19	6

In culture tests from 100 seedlings a Phytophthora fungus, probably *Phytophthora parasitica,* was obtained in a number of cases; and also Fusarium and a yellow bacterium. The last 2 organisms were probably secondary, but the pathogenicity of each is being tested.

A root rot has been observed in several localities on tangerine trees with lesions on the upper exposed or nearly exposed roots, with extension of the infection into the base of the trunk. A species of Fusarium has been found spreading as a thin, whitish superficial web over the infected area. In the

cracks of infected bark, abundant perithecia of a Nectria species also were found. Infected bark of roots shreds off easily. The symptoms at the base of the trunk are not unlike common foot-root symptoms, but without gum. The pathogenicity of the 2 organisms is unknown. The disease has been seen on nothing but native tangerines. It may be a form of dry root rot.

Damping-off of seed-bed plants also was troublesome during 2 successive years in Vicosa. Rangpur lime and Rough lemon were the varieties attacked, and the microörganisms associated were found to be species of Fusarium and Phytophthora. The loss during the first year was calculated at 15 per cent and in the second year 5 per cent. No damping-off appeared during the third year. The disease is apparently common in a great many crops here and cannot be disregarded in connection with seed bed work under the conditions that prevail.

DISEASES OF LEAVES, TWIGS, AND FRUIT

Scab.—This can be considered a major disease here. Moderate to heavy infection has been found on the following varieties: Genoa lemon, Rough lemon, Sweet lemon, King tangerine, West Indian lime, Rangpur lime, and the Bitter Sweet. In the case of the last two, 100 per cent infection of the plants has been observed in nursery rows. In the case of the Rangpur, no mature tree has yet been seen that is not severely scabbed on foliage, twig, and fruit. Grapefruit and Sweet orange have not been found affected with scab in Vicosa, but the latter, in the neighboring State of São Paulo, is subject to the disease.

Melanose.—This disease has been seen mostly in old, closely planted patches of seedling orange trees, on the fruit and on the foliage. In the college planting of the "Marfim" lemon it has been especially noticeable, occurring on fruit, foliage, and twigs. On dead twigs in the interior of the trees, pycnidia of the Phomopsis stage have been encountered. Though Phomopsis is considered one of the chief causes of decay of exported Brazilian fruit, no observations have yet been made as to the importance of this phase of the disease here in the interior.

Anthracnose.—This disease, like scab, seems to be present in Minas throughout the year, being found on young twigs and new foliage, in seed beds and in the nursery row, buds, and flowers, young and mature fruits, on mature foliage, but never as a disease resulting in severe losses. Seed-bed infection has reached 5 per cent, while on budded nursery stock, the maximum loss is 10 per cent. The varieties on which a Colletotrichum infection has been found are: grapefruit, shaddock, Rangpur lime, Sweet orange, and particularly Sweet lemon and Genoa lemon. Dying of large areas on leaves, from the tip or spreading from the margin, has been found, especially on Sweet lemons with which is constantly associated the anthrac-

nose organism, *Colletotrichum gloeosporioides*. The disease has never been found causing infection of more than a small percentage of the leaves on the trees. Without doubt there exists here more than one strain of Colletotrichum, since, in cultures, there have appeared distinct forms of *C. gloeosporioides*. No severe cases of dying back have been seen. The disease can be considered as of minor importance, except under special conditions favoring its development. Anthracnose rot of fruits of the lemon and orange and tangerine has been encountered every year, but it is of minor importance.

Felt.—Wherever trees are planted excessively close together it is common to find a brownish white, felt-like fungus living as thick covering over a scale insect on leaves, twigs, or fruits. No direct serious damage seems to be sustained. The felt seems to be very troublesome to remove from the peduncle end of the fruit during packing operations. Dr. Eugenio Rangel has determined this to be *Septobasidium albidum* Pat. and uses the same name for another felty fungus that is of an entirely different color and consistency, which may live unassociated with scale. The latter must be a distinct species. It forms thin, brownish red plates, the center often densely webby. It commonly ties 2 or more adjacent leaves together and is found on twigs and fruits as well as foliage. Like the former it is troublesome to remove from fruit. It is of minor importance.

LEAF DISEASES

Leaf-spotting has been found on old, neglected orange trees with which a fungus of the genus Phyllosticta has been associated. No apparent serious damage to the trees has been observed. Dr. A. Sacca has reported *P. hesperidearum* as causing leaf-spotting in São Paulo and the fungus found here resembles this.

Spotting of leaves of seedling grapefruit trees at Vicosa accompanied by dropping of the affected leaves is often found. Young, as well as mature, foliage is attacked, the leaves yellowing as the spots enlarge or coalesce. The organism associated has been identified by A. Puttemans as *Ascochyta citri* Penz. The disease is of minor importance.

Algal leaf-spot is sometimes encountered on mature foliage. The organism resembles the common *Cephaleurus mycoidea*. The damage is inconsequential. Defoliation has never been seen.

Sooty mold is frequently found here accompanying scale insects. Curling of heavily coated leaves has been observed in nursery rows, this stage really occasioning some damage.

Black Melanose is common in nursery rows under poor growth conditions, but there seems to be no evidence of damage other than the yellowing of the leaves attacked, and this may be due to something else.

FRUIT ROTS

Blue and Green Mold.—Both of the common species of Penicillium, *P. digitatum* and *P. italicum,* are to be found throughout the citrus fruit season here, present on oranges, limes, lemons, grapefruits, citrons, and tangerines. Judging from the nearly complete local lack of understanding of the necessity of care and method during the processes of picking and handling the fruit, the losses due to these fungi could be very high only where Minas fruits entered in commercial channels.

Sour Rot.—Perhaps equally as common as the *Penicillium* spp. has been a species of Oospora, which seems to be identical with *O. citri-aurantii* during the 4 crops so far observed. Bruised, especially fallen, fruit rapidly becomes a watery disagreeable-smelling decayed mass on exposure to the fungus. The organism is frequently encountered producing decay of numerous fruits still on the tree, but in all cases the fruits show positive evidence of previous injury, generally in the form of insect puncture. Artificial inoculations have shown that the fungus produces rapid decay under our conditions.

Aspergillus Rot.—Rot produced by *A. niger* has been observed on bruised and fallen fruits during 2 crops, but not so often as decay occasioned by the already mentioned organisms. It would probably be only a minor cause of fruit rot in transit.

Alternaria Rot.—Decay of satsumas and sweet lemons by an Alternaria species has been observed in one crop season involving only a small amount of fruit.

Fusarium Rot.—Decay of mature limes, principally at the button end, by a Fusarium species also has been observed during the last crop, the percentage of infection being small. In most cases the decaying fruits lay on the ground under the trees, although some fruits with small circular brownish lesions at the stem end were still attached, falling readily when touched.

ENTOMOGENOUS FUNGI

There seem to be 5 equally common fungi parasitic on scale insects of citrus in this region. They may be found throughout the entire year and are important factors in the control of these insects. Vera K. Charles has courteously given the following identifications for them: *Cephalosporium lecanii* Zimm., *Tuberculariа coccicola* Stev., *Myriangium duriaei* Mont., *Podonectria* sp., and *Microcera* sp.

ESCOLA SUPERIOR DE AGRICULTURA,
VICOSA, MINAS GERAES, BRAZIL.

TRANSMISSION OF THE CRINKLE DISEASE OF STRAWBERRY[1]

E. K. VAUGHAN

(Accepted for publication, Oct. 15, 1932)

The crinkle disease of strawberry was recently described by S. M. Zeller and the writer.[2] It was then stated that the disease has the general appearance and behavior of a virosis, being apparently systemic, since runner plants from a diseased parent show disease symptoms and those from a healthy parent appear normal. During the winter of 1931–32 experiments were carried on in the greenhouse to determine the infectious nature of the disease and possible means of transmission.

The strawberry leaf aphid, *Myzus fragaefolii* Cockerell, has previously been shown to transmit yellows[3] and witches' broom,[4] and it was thought the same aphid might also serve in the transmission of the crinkle disease. These aphids accordingly were colonized on 3 strawberry plants of the Marshall variety. All 3 plants (designated as A, B, and C) were greenhouse-grown and showed distinct symptoms of the disease. Twenty aphids were then transferred to each of 5 healthy plants of the same variety, records being kept of the plant from which they were taken. They were allowed to feed here for one week and then removed. The results of these transfers are shown in table 1.

TABLE 1.—*Results of transmission by the strawberry leaf aphid, Myzus fragaefolii*

Plant from which aphids were transferred	A	B	C	Total
Number of plants to which aphids were transferred	19	23	8	50
Number of plants which developed symptoms of crinkle	15	20	7	42
Percentage of plants which developed symptoms of crinkle	78.94[a]	86.95[a]	87.50	84.00
Number of plants on which symptoms of crinkle later disappeared	11[a]	11[a]	1	23
Percentage of plants on which symptoms of crinkle later disappeared	73.33	55.00	14.28	54.76

[a] One plant died after showing symptoms of crinkle.

[1] Published as Technical Paper No. 191 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

A resumé of a thesis presented in partial fulfillment of the requirements for the master's degree from the Oregon State Agricultural College.

[2] Zeller, S. M., and E. K. Vaughan. Crinkle disease of strawberry. Phytopath. 22: 709–713. 1932.

[3] Plakidas, A. G. Strawberry xanthosis (yellows), a new insect-borne disease. Jour. Agr. Res. 35: 1057–1090. 1927.

[4] Zeller, S. M. Preliminary studies on witches' broom of strawberry. Phytopath. 17: 329–335. 1927.

It is of interest that in 23 of the plants more or less marked symptoms appeared and later disappeared so as to make diagnosis practically impossible, while in 17 plants typical symptoms have persisted. (Figure 1.) No investigation has yet been carried on to explain this disappearance of symptoms, nor have these plants, without apparent symptoms, been tested as carriers of the virus.

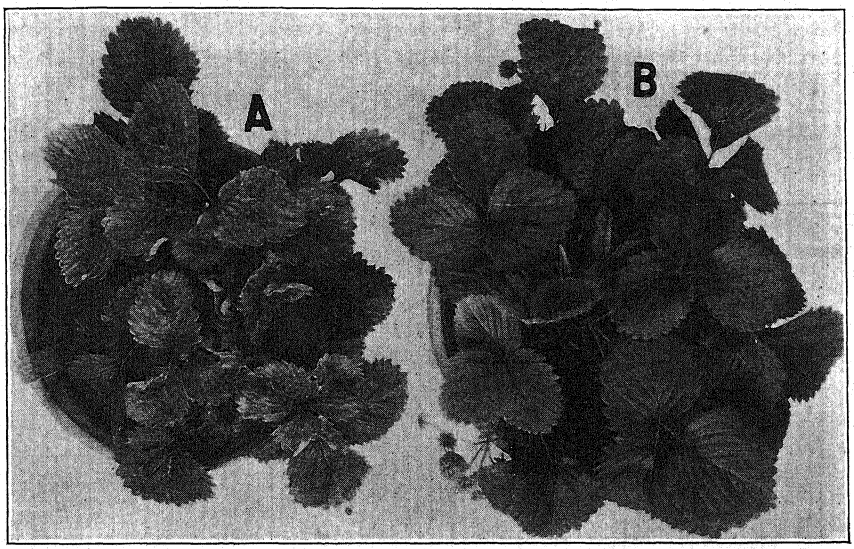


FIG. 1. Marshall strawberry plants of the same age. A. Plant to which crinkle has been transmitted through *Myzus fragaefolii*. B. Healthy plant.

Nonviruliferous aphids also were transferred to healthy plants. These virus-free aphids were obtained by placing adult, agamic female aphids in Petri dishes without food material. Here they were observed at intervals of a few hours until a few young were obtained, which, having had no opportunity to feed were supposedly free of virus contamination. After being allowed to colonize and multiply on a healthy Marshall plant for several weeks, 20 of these aphids were transferred to each of 10 other healthy Marshall plants and allowed to feed for one week. None of these plants showed any symptoms of crinkle after 28 weeks.

Plants of the Ettersburg No. 121 variety, which showed much less pronounced symptoms than the variety Marshall, were thought to have the crinkle disease. Nonviruliferous aphids were colonized for 14 days on a plant of Ettersburg No. 121 having these less marked symptoms. Twenty aphids from the plant were then transferred to each of 10 healthy Marshall plants, where they were allowed to feed for a week. Five of the 10 Mar-

shall plants developed crinkle; in each case only the "pin-point" chlorotic areas described in a previous paper[5] have thus far appeared.

The above experiments have a 4-fold significance: they show, first, that *Myzus fragaefolii* does not produce crinkle directly, but that the viruliferous aphids may act as vectors for the transmission of the infective principle; second, that the infective principle of the crinkle disease of strawberry does not pass from the adult aphids to the young, the first instars; third, that crinkle may be transmitted from one variety to another; *e.g.*, from Ettersburg No. 121 to Marshall; and fourth, that after the first shock from the virus some plants may apparently recover.

Since it is not always possible to detect the symptoms of crinkle in young plants, one could not state definitely that all of the plants used were entirely free of crinkle before having the aphids transferred to them. However, all of them were carefully selected, and as far as could be detected were entirely free of the disease. Of 104 selected Marshall plants obtained in December, 1931, not one has developed crinkle during 34 weeks, unless it was inoculated, while of 24 from this same selection used in the above experiments with viruliferous aphids 14 developed the disease.

No attempts were made to transmit the crinkle disease by means of other insects. *Myzus persicae* Sulzer and an aphid from tobacco were not successfully colonized on strawberry, and the strawberry-root louse, *Aphis forbesi* Weed, was not induced to reproduce during the winter. Mechanical transmission by means of (1) graftage, (2) pricking through diseased leaves into healthy ones, (3) leaf mutilation, and (4) use of extracts from diseased leaves proved unsuccessful.

These experiments were carried on under the direction of Dr. S. M. Zeller, Plant Pathologist, Oregon Experiment Station, whose advice and assistance are sincerely appreciated.

SUMMARY

In this paper are given results of experiments that indicate (1) Crinkle disease of strawberry is caused by a virus, which may be transmitted by the strawberry leaf aphid, *Myzus fragaefolii*. (2) The infective principle does not pass from the adult aphids to the first instars. (3) The disease can be transmitted by aphids from the Ettersburg No. 121 to the Marshall variety. (4) Some plants may recover after the first shock of inoculation. No infection was obtained by such mechanical inoculations as graftage, by leaf mutilation, or by the use of extracts from diseased leaves.

[5] Zeller and Vaughan, *loc. cit.*

OCCURRENCE OF WATERMELON MOSAIC

M. N. WALKER

(Accepted for publication, Sept. 28, 1932)

The natural occurrence of watermelon mosaic was observed early in April, 1932, in Polk County, Florida, near Fort Meade, by the County Agent, F. L. Holland. At that time only 2 or 3 diseased plants were observed in each of 2 fields approximately ¼ mile apart. On April 27, when the writer first inspected these fields, from 20 to 26 affected plants were found in each of several areas along the edges of the fields. Because of the variation in the stages of development of the disease as between plants, it appeared that only 1 or 2 early infected plants in each case had served as the source for secondary infection. The plants outside of these infected areas were in good condition, the vines being nearly full-grown and bearing melons weighing about 15 pounds each.

The diseased vines were very conspicuous because the tips of the runners and a proliferation of shoots from around the crowns protruded stiffly above the general level of the vines, giving what was described by the grower as a "petunia-like" appearance. These tips and shoots showed a shortening of the internodes, resulting in crowding of the young leaves, which appeared somewhat stunted and more rolled than normal. Some mottling was evident on these leaves, the mottling being of a diffuse type, consisting of irregular yellow areas only slightly lighter in color than the normal light green of the young leaves. On some plants these young leaves showed extreme malformation, being reduced in a few cases to almost nothing more than the mid-rib. Older leaves showed a more conspicuous mottling in which dark green raised areas appeared, but the mottling was less characteristic than the severe malformation that occurred on many of the leaves (Fig. 1).

In addition to the crowding of the young leaves on the tips there was also a crowding of flowers and flower buds. Some flowers on the more severely infected plants showed abnormalities of the petals and floral parts, such as unequal lengths and a greenish cast of the petals. The stamens and stigmas were distorted and unequally developed in a few cases. Necrosis and shedding of blossoms occurred before the flowers matured. No melons were set on such plants and the growth and spread of the vines were no more than half of normal vines or vines recently infected. Melons were found on some plants of the latter type, but in most cases they were disfigured by a pronounced mottling of slightly raised, dark green areas. The most pronounced mottling occurred on melons of considerable size

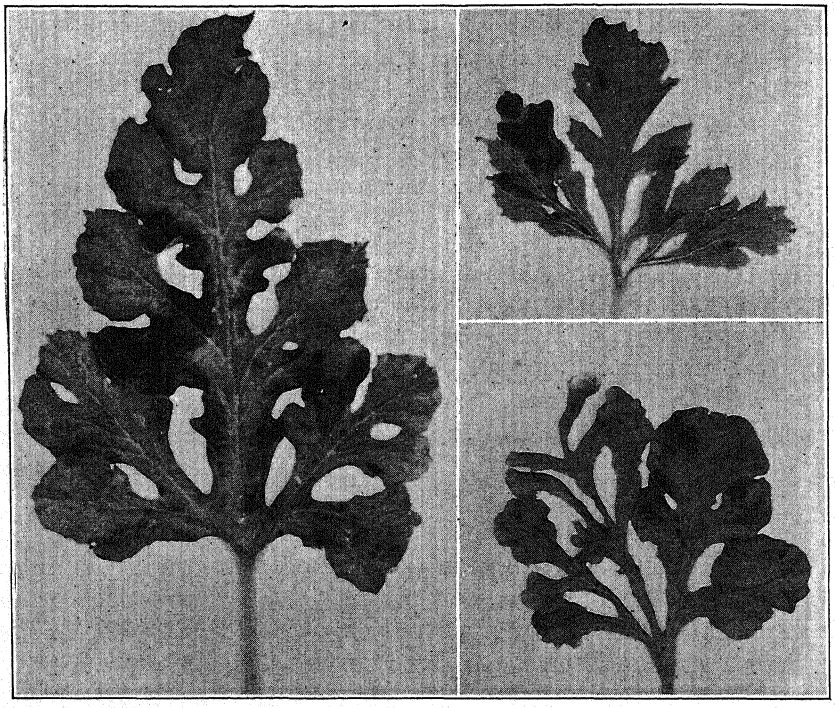


FIG. 1. Leaves from mosaic watermelon plants showing different degrees of mottling and malformation.

(Fig. 2), although small melons were mottled and often malformed. Some of the malformed melons showed pale depressed areas, resembling somewhat the pale areas on the leaves which appear drawn and hard from the concentration of veins. Small blotches of a somewhat paler green than those on other parts of the melon appeared in these areas.

When the fields were inspected a week later, the petunia-like character was less conspicuous, possibly because of the extremely dry weather obtaining at the time. There was some evidence of further spread of the disease, however, and infected plants were found scattered over a 30-acre field, which showed the worst early infection. This field showed an average of 1 per cent infected plants, most of which were concentrated in 4 areas in different parts of the field. Comparatively few melons were found in these areas and most of them were disfigured. Another field of 70 acres showed one badly diseased area with an occasional infected plant near-by. These 2 fields had survived the cold of early March, and were further advanced than a third near-by field of about 15 acres that was planted after the cold spell. A single diseased plant was found in the latter. Another field of about 30

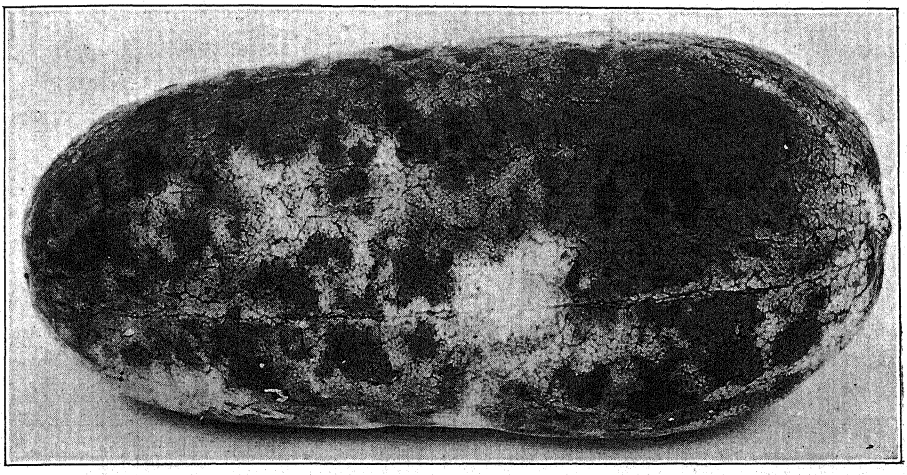


FIG. 2. Half-grown melon showing severe disfiguration as a result of mosaic infection.

acres, 3 miles from the other 3 fields, showed an area that contained about 20 definitely mosaic plants and a few others that were probably infected with mosaic, although they showed nothing more than a general yellowing. All 4 fields were planted to the Tom Watson variety of melons.

The agency of transmission has not been determined but there was an unusually heavy infestation of aphids, *Aphis gossypii,* in the state during the spring, and opportunities for the spread of mosaic by this insect were abundant. A close survey was made of the immediate vicinity of the field showing the greatest number of mosaic plants and no diseased wild plants were found, but 4 species of plants that might serve as wild hosts were found abundantly in and around the diseased field. These plants were 2 species of Physalis: *P. ciliosa,* a perennial, and *P. angulata,* an annual; one species of Phytolacca, *P. rigida;* and an unidentified species of Asclepias. No cultivated crops other than sweet potatoes had been grown in the vicinity of these fields during the preceding year.

This discovery of watermelon mosaic in the field was surprising since no reports have been found in literature on the natural occurrence of watermelon mosaic. One of the oldest growers in Polk County stated that he had seen nothing of similar nature during his 25 years' experience as a watermelon grower in that vicinity. Furthermore, only 3 reports have been found on the successful transmission of mosaic to watermelon plants. Jagger reported[1] the infection of 2 watermelon plants of the variety Cole's

[1] Jagger, I. C. Experiments with cucumber mosaic. Phytopath. 6: 148–151. 1916.

Early with a virus from cucumber plants. Porter described[2] a mosaic disease of cucumbers caused by cucumber Virus 2 which he successfully transmitted to several varieties of watermelons. Porter and Melhus reported[3] the F_1 generation of certain watermelon-citron crosses to be highly susceptible to mosaic.

It is possible that this occurrence of watermelon mosaic[4] was the result of an unusual combination of certain factors which may not be properly combined again for several years. However, since the disease showed such potentialities for injury, a close watch should be kept for its recurrence.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

[2] Porter, R. H. The reaction of cucumbers to types of mosaic. Iowa State Coll. Jour. Sci. 6: 95–129. 1931.

[3] Porter, D. R., and I. E. Melhus. The pathogenicity of *Fusarium niveum* (E F S.) and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad). Iowa Agr. Exp. Sta. Res. Bul. 149. 1932.

[4] No report of the occurrence of this disease has been received during the 1933 season.

PHYTOPATHOLOGICAL NOTE

Humus Extract Agar favorable for Oospore Production in Pythium.—The frequent absence of the sexual stage on ordinary culture media is a widely recognized handicap in identification of certain species of Pythium. Working with a collection of *P. arrhenomanes* Drechsler, from root rot of sugarcane, the writers tested a large number of ordinary and special laboratory media with, in some cases, variation in temperature and pH, as well as the effect of mixing with bacteria and molds. Never more than 24 per cent of the strains produced oospores. The latter, when present, were usually more abundant and normal in appearance on the grated carrot agar suggested by Miss Johann.[1] This was especially noticeable in the culture of *P. arrhenomanes* studied by her and used also in these tests.

What seemed to be oospores of one of the recalcitrant strains had been observed in infected cane roots in an advanced stage of decay. Therefore, soil and humus extracts (so-called humic acid) were added to the carrot medium. Transfers to this have resulted in the development of normal oospores by 95 per cent of the isolates, making possible their definite identification. Although a given culture has not fruited every time, it always has upon one or more replatings.

The humic acid used largely by the writers was extracted from a local woodland soil according to the method described by Burk, Lineweaver and Horner.[2] Satisfactory sporulation, however, has been obtained in the presence of humus extracts from two other widely different sources, *viz.*, from a fertile garden soil and from decomposed filter-press cake, a 50 per cent organic residue from the clarification of sugarcane juice. The humic acid at the rate of 50 p.p.m. (organic content) is added to cornmeal agar, which is then tubed and the grated carrot added to each tube before autoclaving, as suggested by Johann. Carrot or other vegetable tissue is usually essential. Synthetic humic acids prepared from dextrose with and without added iron, and ferric citrate alone, did not stimulate oospore development. —R. D. RANDS and ERNEST DOPP, Division of Sugar Plant Investigations, Bureau of Plant Industry, Washington, D. C.[3]

[1] Johann, Helen. Grated carrot agar favorable for studies of Pythium. (Note) Phytopath. 18: 710. Aug. 1928.

[2] Burk, D., H. Lineweaver, and C. K. Horner. Iron in relation to the stimulation of growth by humic acid. Soil Sci. 33: 413–453. June 1932.

[3] The writers are indebted to Messrs. Burk, Lineweaver, and Horner for helpful suggestions, a few of the humic acids tested, and for determinations of organic matter in others.

A Ringspot-like Virus Disease of Red Clover. In September, 1931, a virus disease of red clover, *Trifolium pratense,* differing from the mosaic disease usually described, was seen in a small planting in the rear of the Kentucky Agricultural Experiment Station greenhouse. A number of plants in each of 4 clover strains, Kentucky, Oregon, Wisconsin, and French, in this area, were affected by this disease.

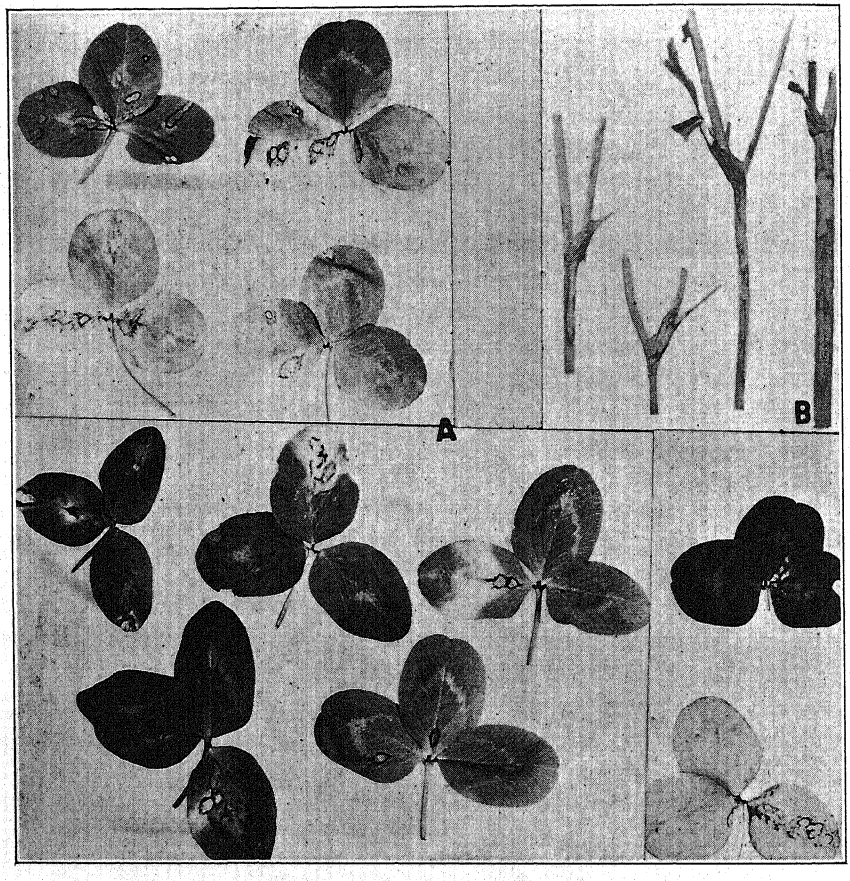


FIG. 1. Ringspot-like virus disease of red clover. A. On the leaflets yellow, circular areas of various sizes with necrotic borders develop. Some leaflets thus affected lose nearly all of the green color and assume a lemon-yellow appearance. B. On stems appear yellow ring-like patterns with brownish borders.

Small, irregular to circular, yellowish spots from a few millimeters to 2 cm. in diameter, with well-defined necrotic borders were observed. (Fig. 1, A.) The chlorosis or yellowing in some spots spread out irregularly

beyond the necrotic border and sometimes one or more leaflets were entirely yellowed. In these the necrotic, ring-like areas were always clearly visible. Long, narrow, irregularly oval, yellow areas with necrotic borders were present on some of the stems of affected plants. (Fig. 1, B.) Usually only a few leaves of a plant were affected and the vigor of the plant did not seem lowered.

The spots resembled those of yellow ringspot of tobacco and a row of tobacco affected with yellow ringspot grew a few feet from the clover. Attempts to transmit the clover disease to 27 Turkish tobacco plants by rubbing juice of affected clover on the leaves by means of sterile swabs were unsuccessful. Aphids were transferred to Turkish tobacco plants from ringspot clover plants. Although a number of transfers were made to the same plants, the insects always died within 4 or 5 days. Even though no symptoms developed in these tobacco plants, inoculations were made from them to healthy tobacco plants, but with negative results.

The garden bean, *Phaseolus vulgaris,* is susceptible to tobacco ringspot. Five bean plants inoculated with juice from diseased clover developed no recognizable symptoms. The proximity of the affected clover plants to tobacco plants affected with yellow ringspot and the close correspondence in symptoms suggest an identical cause, yet inoculation tests leave this question open.

Diseased clover plants were marked and seed harvested in October. Two hundred and fifty-five seeds from 5 plants were sown in flats from which 165 seedlings developed. No symptoms of disease were observed in any seedlings. This disease was seen for the first time in 1931 and only in a small plot. The writer was unable to find the disease in extensive strain tests of red clover on the experiment station farm in 1931–1932.—E. M. JOHNSON, Kentucky Agricultural Experiment Station, Lexington, Ky.

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THE THERMAL DEATH RATE OF TOBACCO-MOSAIC VIRUS

W. C. PRICE[1]

(Accepted for publication July 29, 1933)[2]

INTRODUCTION

The relation between temperature and inactivation of plant viruses is useful in allowing separation of groups of viruses. Resistance of tobacco-mosaic virus to high temperature has been studied by a number of investigators. For the most part, these studies were limited to determinations of the maximum temperature to which this virus might be heated without losing its infectivity. Very little is known about the rate of inactivation of tobacco-mosaic virus at any given temperature or of the effect of different temperatures for a given length of time. The lack of a suitable and adequate method for measuring virus concentration has made it difficult and tedious to study such factors as the gradual diminution in virus concentration due to heating. Because of the recent development of the local lesion method by Holmes (5) and a modification of this method (13), it has been possible to make a quantitative study of the effect of heat on the virus of tobacco mosaic. The purpose of this paper is to present the results of this study.

REVIEW OF LITERATURE

Mayer (10) reported that the infectivity of sap of diseased plants was not perceptibly changed by continued heating at 60° C., that it was weakened by heating to 65°–70° C., and that it was completely destroyed by heating for several hours at 80° C. Iwanowsky (6) found that the infectious property of juice from mosaic-diseased plants was completely lost when heated to temperatures near the boiling-point. Beijerinck (2) stated that tobacco-mosaic virus was inactivated by boiling or when kept for a short time at 90° C. Raciborsky (14) reported that sap from diseased plants did not lose its infectivity when heated for five minutes at 62° C. or for one minute at 100° C., but that its infectivity was completely destroyed when heated for fifteen minutes at 100° C. Koning (9) con-

[1] This work was begun while the writer was employed by the Boyce Thompson Institute for Plant Research, Yonkers, New York.

[2] Published at the expense of The Rockefeller Institute for Medical Research, out of the order determined by the date of acceptance of the manuscript.

cluded that virus diluted 1:10 with water and heated in closed tubes remained infectious when the heating process was carried on for ten minutes at 80° C., five minutes at 90° C., or five minutes at 100° C., although it was considerably weakened by heating for five minutes at 100° C. Woods (16) stated that boiling did not completely inactivate tobacco-mosaic virus in expressed juice. Allard (1) found that the thermal death point of tobacco-mosaic virus in freshly expressed juice was in the neighborhood of 90° C. for ten minutes and that the virus was quickly destroyed at temperatures above this point. He also showed that virus in dried material required much more heat for inactivation than that in solution.

Mulvania (12) found that tobacco-mosaic virus was completely inactivated when heated for ten minutes at 90° C. and was considerably weakened when heated for the same length of time at temperatures slightly below this. He observed, on the other hand, that a similar sample of virus was not completely inactivated when heated for as long as three days at 80° C. Mulvania did not observe any correlation between the water content of a virus sample and its thermal death point. McKinney (11) found that the thermal death point of virus in freshly expressed juice of mosaic-diseased plants was between 88° C. and 90° C. for ten minutes and that the thermal death point of a similar virus sample in a water dilution of 1:100 was between 82° and 84° C. for ten minutes. He concluded that the thermal inactivation of tobacco-mosaic virus is affected by the concentration of virus in the inoculum and also by the plant constituents in which the virus is contained. Brewer *et al.* (4) found that tobacco-mosaic virus (Johnson's tobacco virus 1) in freshly expressed juice of diseased tomato was completely inactivated when held at 88°–90° C. for ten minutes, or at 82°–84° C. for twenty-five minutes.

Recently, Johnson and Grant (8) have shown that tobacco-mosaic virus is not always completely inactivated when heated for ten minutes at 90° C. but that the thermal death point is between 90° and 95° C. for ten minutes. They also studied the influence of host plant on thermal inactivation of tobacco-mosaic virus and found that the thermal death point varied as much as 5° C., depending on the host species from which the virus was obtained. Whether this variation is the result of a lower concentration of virus in some hosts, or of the effect of the host juices, was not ascertained.

MATERIALS AND METHODS

The studies reported in this paper were conducted with ordinary tobacco-virus, classified by Johnson (7) as tobacco virus 1 and used by Holmes (5) and Price (13) in their studies on local lesions of tobacco mosaic. The virus samples used for heating experiments were obtained

from diseased plants of *Nicotiana tabacum* L. var. Turkish, which had been infected for 30 or more days. Juice was extracted from such plants by grinding in a meat chopper and pressing by hand through one thickness of cheese-cloth.

Method of heating virus samples. In the studies where it was necessary to maintain a uniform temperature for only a short period of time (periods up to two hours), the virus samples were immersed in an electrically heated and stirred water bath, held within 0.2° C. of the desired temperature. At the end of the heat treatment, the virus samples were plunged into cold water. In such tests the virus samples were placed in containers prepared from glass tubing of 3–4 mm. bore and in 25–30 mm. lengths. At the time of testing a virus sample, one end of a tube was drawn to a fine point in a flame. Approximately two cc. (one cc. for the one-minute tests) of the sample to be tested were drawn into the tube, one end of which was then carefully sealed by heat. This treatment did not measurably decrease the infectivity of the virus sample, as was shown by tests conducted with samples immediately after preparation.

Tests were made to determine the length of time required for a virus sample to reach the temperature of the water bath in which it was placed. These tests were made by the use of a thermocouple attached to a galvanometer. The results of the tests showed that from 40 to 45 seconds were required for the temperature of the solution in the tube to reach the temperature of the bath. In 30 to 35 seconds after immersion there was a difference of one degree in temperature of the two solutions; in 25 seconds there was a difference of four degrees in temperature. The tests were made in a water bath held at temperatures of 100° C., 90° C., and 80° C., respectively. In the heating experiments shown below, no allowance was made for the time required for the temperature of the virus solution to reach the temperature of the bath. The time periods shown in the table represent the actual time the tubes were immersed. Thus, a virus solution that was kept for one minute in a bath held at 95° C. was actually maintained at 95° C. for only 15 to 20 seconds, from 95° to 94° C. for ten seconds, and from 94° to 91° C. for ten seconds.

In experiments in which it was necessary to maintain a uniform temperature over a period of time longer than two hours, a slightly different procedure was followed. Approximately three cc. of a virus sample were placed in a tightly stoppered 1 x 10 cm. test tube. Such tubes were kept in electrically heated ovens for the duration of the test. The temperature was taken at the level of the tubes in the ovens. The oven temperature, as a rule, did not vary more than $\pm$ 1° C., but occasionally a variation of as much as 2° C. was observed. In the later experiments the tubes of virus were immersed in a paraffin bath kept inside the ovens.

Method for measuring virus concentration. The method employed by previous workers for testing for presence of virus in samples of heated juice depended upon the appearance of the systemic disease in plants of *Nicotiana tabacum* inoculated with such samples. Usually five or ten plants were used for each sample tested and the results were given in percentage of infection. The appearance of the systemic disease in this host indicates the presence of virus in the inoculum but gives little indication of the relative concentration of virus unless a large number of plants are used. The local lesion method (5, 13) for measuring virus concentration not only differentiates between presence and absence of virus but also allows a reasonably accurate estimation of virus concentration. The method was employed in the studies reported in this paper. Lesions made their appearance in about 36 hours and were usually counted in five or more days after inoculation. The test plants were grown in a greenhouse in which the temperature was not allowed to fall below 68° F.

Some of the experiments reported in this paper were conducted with *Nicotiana glutinosa* L. as the test plant and the remainder with *Phaseolus vulgaris* L. var. Early Golden Cluster as the test plant. Several of the experiments with *N. glutinosa* were duplicated with *P. vulgaris,* thus allowing a comparison to be made between the two test plants. It is of interest to note that there was a close agreement between the results obtained with these two hosts.

In cases in which *Nicotiana glutinosa* was used as the test plant, five leaves of this host were inoculated with the sample tested. Plants used in these experiments were selected for uniformity of leaves at the time of making the test. In those cases in which *Phaseolus vulgaris* was used as the test plant, each virus sample was tested by inoculation of 16 primary leaves, which were almost fully expanded.

EXPERIMENTAL

Thermal death point is usually defined as the minimum temperature that results in complete inactivation after exposure for a definite period of time under specific conditions. A ten-minute period is usually taken as a standard. Similarly, thermal death time may be defined as the length of time required for complete inactivation of a virus sample when exposed to a definite temperature under specific conditions. The writer determined the thermal death point of tobacco-mosaic virus for a ten-minute exposure and for a one-minute exposure and the thermal death time for temperatures of 68°, 75°, 80°, 85°, and 90° C. The details of these experiments are given below.

THERMAL DEATH POINT: TEN-MINUTE EXPOSURE

Seven different experiments were conducted in order to determine the thermal death point (ten-minute exposure) of tobacco-mosaic virus in freshly expressed juice of diseased Turkish tobacco plants. These experiments were conducted at different times of the year and included seven different virus samples. Table 1 shows the results of four experiments in which *Nicotiana glutinosa* was used as a test plant. The results of three experiments in which Early Golden Cluster beans were used as test plants are given in table 2. These results indicate that there is a slight variation in thermal death point of different samples of virus, the minimum thermal death point observed being 91° C., the maximum 93° C. On the other hand, it is possible that this seeming inconsistency resulted from variation in test plants and the failure to detect minute quantities of virus in some instances rather than from any variation in virus samples. It is concluded from the results of these experiments that the thermal death point of tobacco-mosaic virus in freshly expressed juice of mosaic-diseased tobacco plants is 93° C. for ten minutes.

The results given in tables 1 and 2 also show that partial inactivation of virus occurs at temperatures well below the thermal death point and that this inactivation increases with rise in temperature. In all cases observed, a temperature of 87° C. (six degrees below the thermal death point) caused a significant decrease in virus concentration.

Effect of water dilution on thermal death point: ten-minute interval. It is well known that the thermal death point of various organisms is influenced by the nature of the dispersing medium and the number of organisms in the sample. It is, therefore, of interest to know whether the thermal relations of tobacco-mosaic virus, as well as of other viruses, are influenced by such factors. It is already known that the thermal death point of tobacco-mosaic virus is appreciably lowered by dilution (11). Several experiments were undertaken in order to obtain more detailed information regarding the effect on thermal death point of dilution with water. Freshly extracted juice of mosaic-diseased plants diluted 1:20 with water was used in these experiments. Table 3 shows the results of six tests with juice diluted 1:20 with water. With three of the virus samples tested, the thermal death point was found to be 88° C. for ten minutes; with one sample, the thermal death point was 87° C.; with the remaining samples 86° C. These results show that dilution with water significantly lowers the thermal death point of tobacco-mosaic virus.

It is also of interest, in connection with these experiments, to note that, as in the case with undiluted juice, temperatures considerably below the thermal death point cause a marked decrease in virus concentration and that the degree of inactivation increases with rise in temperature.

Effect of dilution with healthy tobacco extract. Since it has been shown that the thermal death point of tobacco-mosaic virus is significantly lowered by dilution with water, it is of interest to know whether this decrease is the result of the decrease in virus concentration or of a decrease in total solids or of both. Such a question is difficult to answer until it is possible to work with a purified virus solution. Some information regarding it has been obtained, however, by using juice of healthy tobacco plants as a diluting medium. In such a dilution, the concentration of virus can be considerably lowered without any appreciable decrease in solid content of the solution. Such a solution is not strictly comparable with undiluted juice of mosaic-diseased plants, since the presence of virus in the plant no doubt alters the properties of the juices of such a plant. It does, however, allow a rough comparison of the effect of dilution of virus and dilution of solids in the dispersing medium.

Thermal death point determinations were made with juice of mosaic-diseased plants diluted 1:20 with juice of healthy tobacco plants. The failure of control plants to produce lesions when inoculated with juice from these healthy plants proved that the juice used for dilution was free of any appreciable quantity of tobacco-mosaic virus. The results of five tests with virus in freshly extracted juice of mosaic-diseased plants diluted with healthy juice are given in table 4. In two tests, the thermal death point of virus in this dilution was found to be 91° C.; in three tests, 90° C. A comparison of these results with those presented in tables 1, 2, and 3 shows that the thermal death point of virus diluted 1:20 with juice of healthy tobacco plants is two degrees lower than that of undiluted juice and three degrees higher than that of virus in juice diluted 1:20 with water. It is, therefore, concluded that the thermal death point of tobacco-mosaic virus is influenced by the concentration of virus as well as by the nature of the dispersing medium.

An interesting phenomenon is clearly shown by comparison of tables 1, 2, 3, and 4. Although the concentration of virus in the samples used for tests shown in table 4 (juice diluted 1:20 with healthy tobacco juice) was reduced 20 times, the number of lesions produced upon leaves of *Nicotiana glutinosa* by inoculation was only slightly less than that produced by inoculation with undiluted juice. The cause of this phenomenon is not known. It may be due to the presence in healthy juice of some substance that has an effect similar to that of norit or talc, as reported by Vinson and Petre (15).

Effect of filtration on thermal death point. Since the results of previous experiments showed that the thermal death point of tobacco-mosaic virus is influenced by the concentration of virus and by the concentration

TABLE 1.—*Effect of exposure of tobacco-mosaic virus to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
86	154[a]	440	1000	528	2122	530
87	104	777	657	367	1905	476
88	138	491	516	239	1384	346
89	96	209	116	435	856	214
90	69	24	10	108	211	53
91	6	0	0	18	24	6
92	1	0	0	0	1	1
93	0	0	0	1	1	1
94	0	0	0	0	0	0
95	0	0	0	0	0	0
Unheated controls	1812	2700	2650	1236	8398	2099

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

of solids in the dispersing medium, it appeared that further information regarding this phenomenon might be obtained by studying the effect of filtration. Although it is well known that tobacco-mosaic virus is infectious after passage through a Berkefeld filter candle, most workers believe that filtration reduces the concentration of virus. No one has yet determined to what degree filtration reduces virus concentration. Juice from mosaic-diseased tobacco plants was diluted 1:20 with water and immedi-

TABLE 2.—*Effect of exposure of tobacco-mosaic virus to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Total	Average
87	795[a]	2272	2936	6003	2001
88	759	3944	3312	8015	2672
89	123	1712	862	2697	899
90	50	928	512	1490	497
91	0	11	5	16	5
92	0	1	2	3	1
93	0	0	0	0	0
94	0	0	0	0	0
95	0	0	0	0	0
96	.....	0	0	0	0
Unheated controls	3435	3488	5550	12473	4158

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 3.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Test No. 6	Total	Average
77	272[a]	311	91	200	.....	.....	874	219
78	206	200	60	293	.....	.....	759	190
79	146	138	28	109	.....	.....	421	105
80	132	141	65	92	.....	.....	430	108
81	92	247	42	131	23	31	566	94
82	169	86	13	93	95	49	505	84
83	429	123	39	120	12	26	749	125
84	351	183	15	217	14	5	785	131
85	126	114	2	36	4	12	294	49
86	91	35	0	64	0	2	192	32
87	2	6	0	21	0	0	29	5
88	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0
Unheated controls...	334	376	222	369	260	199	1760	293

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

ately filtered through a Berkefeld filter candle of the "N" grade. This solution was kept frozen until needed. Four thermal death-point determinations were made with juice that had been treated in this manner. The results of these tests are shown in table 5. In three of the tests, the thermal death point was found to be 84° C., in one test 85° C. The variation is obviously the result of experimental procedure, since the virus samples were identical in each case. This thermal death point is three degrees below that of unfiltered juice diluted 1:20 with water. It is, therefore, concluded that filtration lowers the thermal death point of tobacco-mosaic virus, although it is realized that freezing also may have altered the resistance of the virus to heat.

Effect of addition of gelatin on thermal death point. In order to obtain more information regarding the influence of concentration of solids on thermal relations of tobacco-mosaic virus, thermal death-point determinations were made with a virus sample to which gelatin had been added. Enough gelatin was added to each solution tested to bring the concentration of gelatin in the sample to two to four per cent by weight. Some slight dilution of the virus sample resulted from this treatment, but the dilution was not significantly large. The results of two tests with undiluted juice of mosaic-

TABLE 4.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with healthy extract) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Total	Average
81	993[a]	1796	194	2100	974	6057	1211
82	378	1675	192	1250	1300	4795	959
83	441	231	98	2303	646	3719	744
84	498	1115	61	456	889	3019	604
85	395	250	70	512	342	1569	314
86	274	1367	20	184	75	1920	384
87	120	397	10	8	0	535	107
88	90	25	3	36	1	155	31
89	4	12	2	11	9	38	8
90	0	2	0	1	0	3	1
91	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0
	906	1735	270	3240	3320		
Unheated controls	1120	1928	470			19932	1533
	2134	1460	162				
	2950		237				
Healthy juice alone	0	0	0	0	0	0	0

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

diseased tobacco plants to which gelatin had been added are given in table 6. In one test with this solution, the thermal death point was found to be 93° C. for ten minutes, in the other test, 92° C. The results of four tests with juice diluted 1: 20 and containing two to four per cent gelatin are given in table 7. In three of these tests, the thermal death point was found to be 88° C., and in one test it was 87° C. for ten minutes. The thermal death points of tobacco-mosaic virus in solutions containing two to four per cent gelatin were identical with those in similar solutions that did not contain gelatin. It is concluded that the addition of this quantity of gelatin has no detectable influence upon the thermal death point.

THERMAL DEATH POINT: ONE-MINUTE EXPOSURE

In all the experiments reported above, a ten-minute interval was taken as a standard in determining the thermal death point. It is of interest to know the thermal death point of tobacco-mosaic virus for a one-minute

TABLE 5.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water and filtered through Berkefeld "N" filter candle) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
76	.....	13[a]	36	14	63	21
77	.....	20	27	36	83	28
78	41	21	11	10	83	21
79	20	12	5	19	56	14
80	15	5	11	5	36	9
81	14	9	14	8	45	11
82	7	9	4	7	27	7
83	2	4	3	6	15	4
84	0	0	0	1	1	0.25
85	0	0	0	0	0	0
86	0	0	0	0	0	0
87	0	0	0	0	0	0
Unheated controls	121	72	127	94		
		35	51	144	822	91
		58	120			

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

TABLE 6.—*Effect of exposure of tobacco-mosaic virus (in undiluted juice containing four per cent gelatin) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Total	Average
88	2246[a]	360	2606	1303
89	775	60	835	418
90	262	33	295	148
91	42	1	43	22
92	5	0	5	3
93	0	0	0	0
94	0	0	0	0
95	0	0	0	0
Unheated controls	2250	1619	3869	1935

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

exposure. In order to determine this, tests were made with undiluted juice of mosaic-diseased tobacco plants and with juice diluted 1:20 with water. Results of these tests are described below.

Undiluted juice. The results of four tests with undiluted juice are given in table 8. In two of these tests, the thermal death point for a one-

TABLE 7.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water and containing four per cent gelatin) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
81	44[a]	132	179	19	374	94
82	21	260	210	40	531	133
83	26	390	124	19	559	140
84	22	52	88	24	186	47
85	7	141	25	8	181	45
86	4	26	11	4	45	11
87	1	9	0	2	12	3
88	0	0	0	0	0	0
89	0	0	0	0	0	0
90	0	0	0	0	0	0
91	0	0	0	0	0	0
Unheated controls	49	519	193	220	981	245

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

minute exposure was found to be 95° C.; in the other two tests, it was 96° C. Considerable inactivation of virus occurred at 92° C. in all four cases, and there was a gradual diminution in virus concentration with rise in temperature. The results indicate, however, that there is a sharper end point in the case of a one-minute exposure than in the case of a ten-minute exposure.

Juice diluted 1:20 with water. Four tests were made in order to determine the effect of dilution on the thermal death point for a one-minute exposure. As in previous experiments, freshly extracted juice of mosaic-

TABLE 8.—*Effect of exposure of tobacco-mosaic virus to different temperatures for one minute*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
92	867[a]	3600	1280	2108	7855	1964
93	338	747	324	1870	3279	820
94	25	251	179	404	859	215
95	0	0	43	3	46	12
96	0	0	0	0	0	0
97	0	0	0	0	0	0
98	0	0	0	0	0	0
Unheated controls	7360	10330	11800	10880	40370	10093

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 9.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to various temperatures for one minute*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
87	1536[a]	648	408	479	3071	768
88	701	336	249	136	1422	356
89	528	71	128	128	855	214
90	208	93	92	11	404	101
91	16	5	6	1	28	7
92	0	0	0	0	0	0
93	.....	0	0	0	0	0
Unheated controls	8600	4400	3306	3516	19822	4956

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 10.—*Effect of exposure of tobacco-mosaic virus to a temperature of 90° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
20 min.	829[a]	7	179	1015	338
30 "	11	1	110	122	41
40 "	30	0	88	118	39
50 "	1	0	57	58	19
60 "	2	0	3	5	2
70 "	0	0	4	4	1
80 "	0	0	0	0	0
Unheated controls	9330	5688	10800	25818	8606

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 11.—*Effect of exposure of tobacco-mosaic virus to a temperature of 85° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
4 hrs.	416[a]	1500	243	2159	720
8 "	64	230	631	925	308
12 "	164	185	408	757	252
16 "	48	6	80	134	45
20 "	9	7	8	24	8
24 "	8	5	24	37	12
28 "	4	0	1	5	2
32 "	0	0	0	0	0
36 "	–	0	–	0	0
Unheated controls	6864	7822	5500	20186	6729

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 12.—*Effect of exposure of tobacco-mosaic virus to a temperature of 80° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Total	Average
1 day	1112[a]	6688	1528	3088	3422	15838	3168
2 days	705	1048	1360	2747	5244	11104	2221
3 "	184	1545	805	2096	1728	6358	1272
4 "	133	444	312	680	860	2429	486
5 "	84	374	112	427	.....	997	249
6 "	18	280	35	640	193	1166	233
7 "	19	78	11	201	101	410	82
8 "	8	19	4	99	89	219	44
9 "	1	6	2	17	21	47	9
10 "	0	0	0	6	23	29	6
11 "	.....	.....	3	1	0	4	1
12 "	.....	.....	0	0	0	0	0
13 "	.....	.....	.....	0	0	0	0
14 "	.....	.....	.....	0	.....	0	0
15 "	.....	.....	.....	0	.....	0	0
Unheated controls	13300	11400	8000	10600	9152	52452	10490

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 13.—*Effect of exposure of tobacco-mosaic virus to a temperature of 75° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
10 days	1466[a]	1866	1600	4932	1644
15 "	263	192	33	488	163
20 "	21	19	20	60	20
25 "	17	11	7	35	12
30 "	21	8	5	34	11
35 "	3	6	0	9	3
40 "	0	0	0	0	0
40 "	0	0	0	0	0
Unheated controls	6044	3916	5964	15924	5308

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

diseased tobacco plants diluted 1:20 with water was used. Results of these four tests are presented in table 9. In all four tests, the thermal death point for a one-minute exposure was found to be 92° C. Partial inactivation occurred at 87° C., the lowest temperature tested.

THERMAL DEATH TIME FOR VARIOUS TEMPERATURES

Undiluted juice. The thermal death time for virus in freshly extracted juice of mosaic-diseased tobacco was determined for temperatures of 90°,

TABLE 14.—*Effect of exposure of tobacco-mosaic virus to a temperature of 68° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Total	Average
4 hrs.	11260	7377	.....	.....	.....	18637	9319
12 "	5250	3700	.....	.....	.....	8950	4475
24 "	7000	4600	.....	.....	.....	11600	5800
2 days	5000	3275	.....	.....	.....	8275	4138
3 "	5350	3303	.....	.....	.....	8653	4327
5 "	2369	811	.....	.....	.....	3180	1590
8 "	4450	1334	.....	.....	.....	5784	2892
10 "	2525	1045	.....	.....	.....	3570	1785
14 "	1635	655	.....	.....	.....	2290	1145
18 "	.....	.....	.....	.....	189	189	189
19 "	905	421	.....	.....	.....	1326	663
20 "	.....	.....	.....	.....	91	91	91
31 "	.....	.....	84	85	.....	169	85
33 "	.....	.....	185	.....	.....	185	185
37 "	.....	.....	.....	.....	12	12	12
39 "	.....	.....	52	.....	8	60	30
40 "	.....	.....	45	.....	.....	45	45
42 "	.....	.....	24	.....	.....	24	24
44 "	.....	.....	.....	.....	0	0	0
46 "	.....	.....	.....	.....	2	2	2
48 "	.....	.....	.....	5	.....	5	5
50 "	.....	.....	.....	5	0	5	3
52 "	.....	.....	0	2	1	3	1
53 "	5	0	.....	.....	.....	5	3
54 "	.....	.....	0	1	0	1	1
56 "	.....	.....	0	37	.....	37	19
58 "	.....	.....	0	4	.....	4	2
60 "	.....	.....	.....	26	.....	26	26
62 "	.....	.....	.....	9	.....	9	9
64 "	.....	.....	.....	5	.....	5	5
66 "	.....	.....	.....	4	.....	4	4
68 "	.....	.....	.....	11	1	12	6
70 "	0	0	.....	4	1	5	1
Unheated controls	17400	17800	.....	.....	.....	.....	.....
	9100	7400	5760	8280	13300	98440	10938
	10600	8800	.....	.....	.....	.....	.....

85°, 80°, 75°, and 68° C. In all cases the virus concentration remaining after heating was measured by inoculation of leaves of Early Golden Cluster beans. Results of tests at these temperatures are shown in tables 10 to 14. Table 10 shows the results of three tests with virus samples held at 90° C.; table 11, the results of three tests at 85° C.; table 12, the results of five tests at 80° C.; table 13, the results of three tests at 75° C.; and

TABLE 15.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to a temperature of 85° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
20 mins.	.....	3[a]	5	114	122	41
30 "	2	0	0	18	20	5
40 "	1	0	2	12	15	4
50 "	1	1	5	10	17	4
60 "	0	0	0	1	1	1
70 "	0	0	0	0	0	0
80 "	0	.....	0	0	0	0
Unheated controls	8600	4155	4225	3200	20180	5045

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 16.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to a temperature of 80° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
1 hr.	2014[a]	430	399	146	2989	747
2 hrs.	493	206	147	200	1046	261
3 "	132	171	291	141	735	184
4 "	76	58	107	45	286	72
5 "	91	93	194	11	389	97
6 "	6	12	220	9	247	62
7 "	5	94	123	2	224	56
8 "	1	12	48	1	62	16
9 "	0	7	45	0	52	13
10 "	5	9	9	0	23	6
11 "	0	0	2	4	6	2
12 "	0	0	1	0	1	0.25
13 "	0	0	0	0	0	0
14 "	0	0	0	0	0	0
15 "	0	1	0	0	1	0.25
16 "	0	0	0	0	0	0
Unheated controls	2014	4186	4784	5500	26990	3856
	4600	2122	3784			

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

table 14, the results of five tests at 68° C. It is concluded from these tests that tobacco-mosaic virus in undiluted juice is completely inactivated when held at 90° C. for 80 minutes, at 85° C. for 32 hours, at 80° C. for 12 days,

TABLE 17.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water) to a temperature of 75° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
12 hrs.	990[a]	100	512	233	1835	459
24 "	355	4	184	40	583	146
36 "	4	0	20	1	25	6
48 "	0	3	68	0	71	18
60 "	0	0	2	0	2	1
72 "	0	0	0	0	0	0
84 "	.....	.....	0	0	0	0
Unheated controls	2448	2915	1228	1511	8102	2026

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 18.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water) to a temperature of 68° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
2 days	.....	906[a]	1070	1976	988
3 "	455	656	456	1567	522
4 "	235	354	576	1165	388
5 "	114	.....	.....	114	114
6 "	171	293	426	890	297
7 "	624	49	62	735	245
8 "	144	61	135	340	113
9 "	37	92	322	451	150
10 "	3	107	19	129	43
11 "	2	2	624	628	209
12 "	167	11	260	438	146
13 "	22	67	394	483	161
14 "	0	0	7	7	2
15 "	5	9	11	25	8
16 "	0	0	0	0	0
17 "	0	5	17	22	7
18 "	0	0	13	13	4
19 "	0	1	8	9	3
20 "	0	0	0	0	0
Unheated controls	3912	760	356	5028	1676

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

or at 75° C. for 40 days. It was not completely inactivated when held at a temperature of 68° C. for as long as 70 days.

Diluted juice. Tests were made to determine the thermal death time at various temperatures for virus in juice of mosaic-diseased tobacco diluted

1:20 with water. Tables 15 to 18 show the number of lesions produced on Early Golden Cluster beans by inoculation with virus heated to these temperatures. Table 15 shows results of four tests with diluted juice held at 85° C.; table 16, results of four tests at 80° C.; table 17, results of four tests at 75° C.; and table 18, results of three tests at 68° C. These results show that virus in freshly extracted juice, diluted 1:20 with water, is completely inactivated when heated to 85° C. for 70 minutes, to 80° C. for 13 hours, to 75° C. for 72 hours, or to 68° C. for 20 days.

CURVES SHOWING RATE OF INACTIVATION

Some of the data obtained in this investigation are plotted in the form of curves and presented in figures 1 and 2. Figure 1 shows the rate of inactivation of tobacco-mosaic virus in undiluted juice held at a temperature of 68° C. Data from which this curve was drawn were taken from table 14. The curve shows clearly the gradual diminution in virus concentration due to heating.

The curves in figure 2 were drawn with time plotted on a logarithmic scale and temperature on an arithmetic scale. They show the time-temperature relation for complete inactivation of virus. The points on the curves represent the times, shown in tables 1 to 3 and 8 to 18, for complete inactivation of virus at the temperatures indicated. With these curves, it is possible to estimate the thermal death time for any temperature between 68° C. and 96° C., or to calculate the thermal death temperature for any period between one minute and 40 days. Although the curve for inactivation of virus in undiluted juice and that for virus in juice diluted 1:20 are similar in shape, they are closer together at the higher temperatures tested than at the lower. This appears to show that dilution alters the thermal death time more at low temperatures than at high temperatures.

DISCUSSION

The results obtained in this investigation are in fairly close agreement with those of other workers. The seemingly higher thermal death point obtained by the writer and also by Johnson and Grant (8) may be due to the use of the rubbing method of inoculation, which allows detection of minute quantities of virus. It has been shown that the thermal death point is influenced by concentration of virus and by concentration of solids in the dispersing medium. It may be that such factors would be operative in the case of virus samples obtained from host plants grown under different environmental conditions. Differences between results observed by various investigators, as well as differences between certain samples of virus used by the writer, may possibly be due to differences in plants from

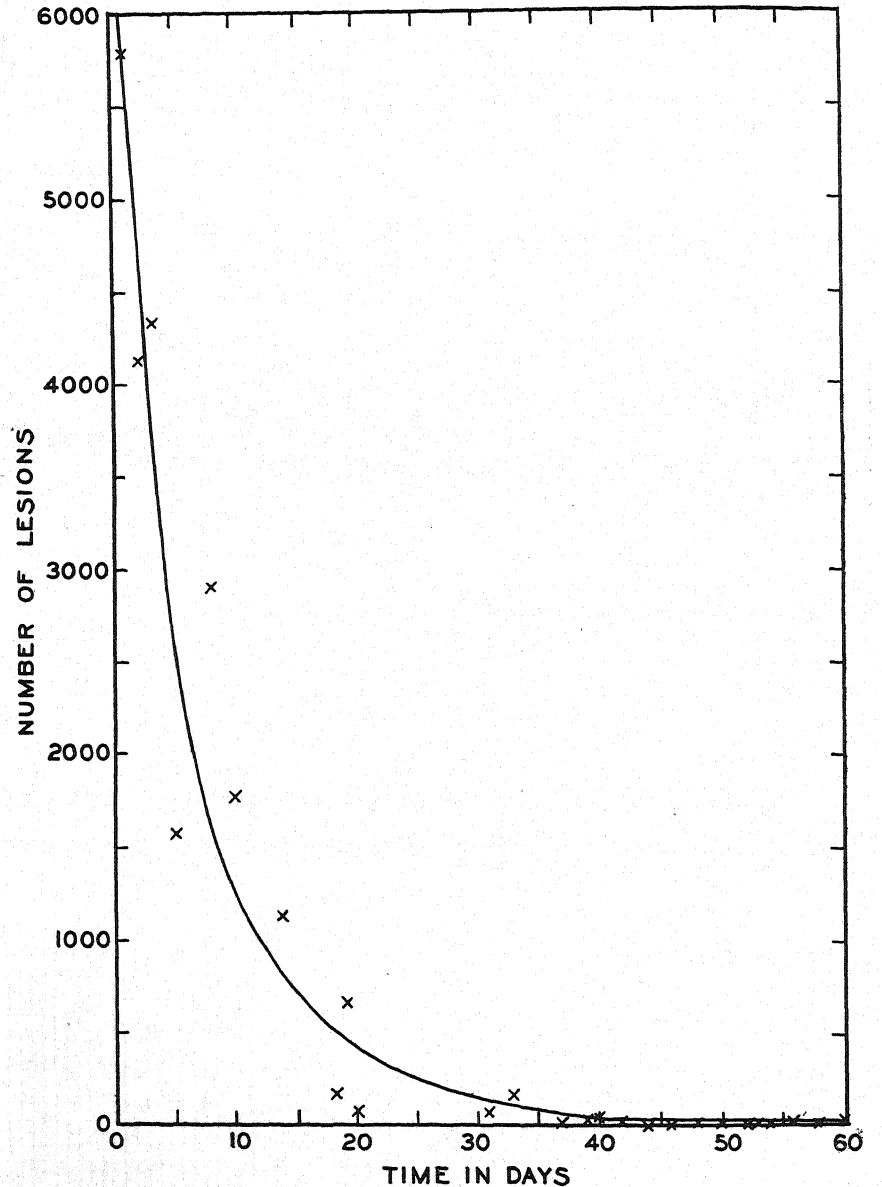


FIG. 1. Curve showing the rate of inactivation of tobacco-mosaic virus (in freshly expressed juice of diseased tobacco plants) when held at a temperature of 68° C. The points on the curve show the average number of lesions produced on leaves of Early Golden Cluster beans by inoculation with virus samples heated for the length of time indicated.

which the virus samples were obtained. It is of importance that such factors as concentration of solids and nature of the dispersing medium be taken into consideration when comparing the thermal death points of different viruses or even of the same virus from different sources.

Another factor that may account for some of the variation obtained in different tests with tobacco-mosaic virus is the difficulty of maintaining a uniform temperature over a considerable period of time. Examination of the curves presented in figure 2 shows that a slight change in temperature causes a significantly large change in thermal death time of the virus. Variation in temperature of as much as one degree C. might result in a considerably shorter or considerably longer thermal death time.

Thermal death-time curves for several thermophilic organisms were published by Bigelow (3). These curves, on semilogarithmic paper, are straight lines except at temperatures below 105° C., where they tend to turn downward. It is of interest that the thermal death-time curves of tobacco-mosaic virus (Fig. 2) resemble those shown by Bigelow for thermo-

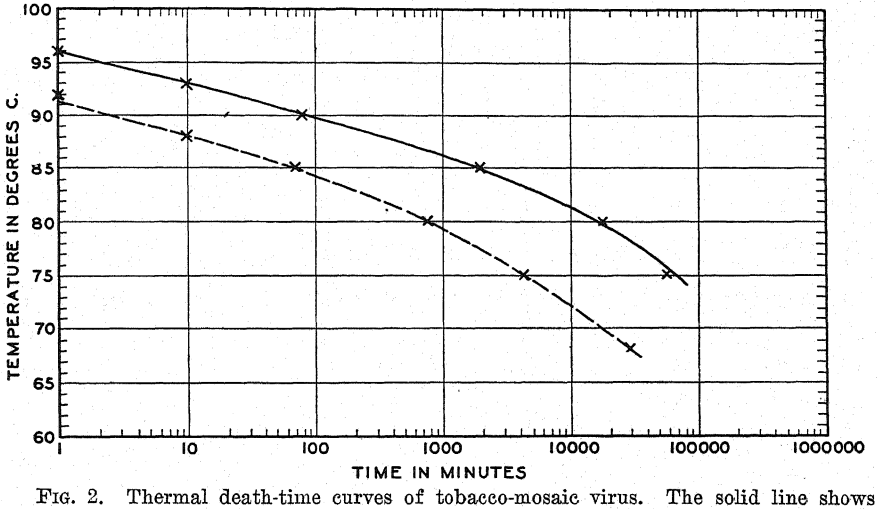


FIG. 2. Thermal death-time curves of tobacco-mosaic virus. The solid line shows the time required for complete inactivation of virus in freshly expressed juice of mosaic-diseased plants at the temperatures indicated; the broken line, the time required for inactivation of virus in juice diluted 1: 20 with water.

philic organisms. These curves for tobacco-mosaic virus are almost straight lines at the high temperatures but bend downward sharply at temperatures below 80° and 85° C.

The virus of tobacco mosaic is more resistant to heat than most of the other plant viruses that have been studied up to the present time. No other plant virus is known to have a higher thermal death point. That it

is far more resistant than are most vegetative bacteria is shown by its ability to withstand the temperature of pasteurization for many days.

SUMMARY

The local-lesion method for measuring virus concentration was used in a quantitative study of the rate of inactivation of tobacco-mosaic virus by heat. It was found that virus in undiluted juice of mosaic-diseased tobacco plants was inactivated in one minute at 96° C., in ten minutes at 93° C., in 80 minutes at 90° C., in 32 hours at 85° C., in 12 days at 80° C., and in 40 days at 75° C. It was not completely inactivated when held for 70 days at 68° C. Virus in juice of mosaic-diseased tobacco plants diluted 1:20 with water was completely inactivated in one minute at 92° C., in ten minutes at 88° C., in 70 minutes at 85° C., in 13 hours at 80° C., in 72 hours at 75° C., and in 20 days at 68° C.

Tables giving measurements of virus concentration after heating for different periods of time at the same temperature and for different temperatures for the same length of time are presented in the text. These measurements indicate that inactivation of tobacco-mosaic virus by heat is a gradual process that goes on more rapidly at first than after partial inactivation has occurred.

Time-temperature curves for inactivation of mosaic virus in undiluted juice and in juice diluted 1:20 with water were drawn on semilogarithmic paper and are presented in the text. They show the time required for complete inactivation of virus at temperatures between 68° and 96° C.

Thermal death-point determinations made with virus in juice diluted with water and in juice diluted with extract of healthy tobacco indicate that inactivation of virus is influenced by concentration of virus and concentration of solids in the dispersing medium.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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LEAF SCORCH OF NARCISSUS[1]

DON B. CREAGER

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A disease that causes premature death of narcissus leaves was brought to the attention of the writer in the summer of 1929 (Fig. 1). In general,



FIG. 1. Narcissus leaves (var. Sir Watkin) destroyed by leaf scorch; natural inoculation.

it appears that the *Leedsii* and *Polyanthus* types of narcissus are most severely affected, but the disease is known to occur also on nearly all of the other types. Among the commercial varieties commonly infected are, Sir Watkin, King Alfred, White Lady, Queen of the North, Laurens Koster, Orange Cup, Early Perfection, and Victoria. The disease occurs also on

[1] Published as a contribution from a cooperative project on the diseases of flowering bulbs maintained by Cornell University Agricultural Experiment Station and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Thanks are due Dr. Freeman Weiss, Pathologist, U. S. Department of Agriculture, for suggesting the problem; Dr. F. A. Wolf, Duke University, and Dr. L. M. Massey, Cornell University, for aid in preparing the manuscript; and to Drs. W. H. Weston, Jr., and D. H. Linder for information concerning the specimen of *Hendersonia curtisii* in the Curtis Herbarium at Harvard University.

the leaves and scapes of amaryllis (hybrids of *Hippeastrum vittatum* Herb.).

Fields or portions of fields of narcissus in which the foliage is severely affected have a burned or scorched aspect, which has given rise to the name "leaf-scorch," as proposed by British phytopathologists, and adopted here.

Leaf scorch was first reported by Aderhold (1) in 1900 on narcissus in Germany, while, apparently, the disease on amaryllis was first noted by Dougherty (3) in 1916. For over a decade the diseases on these two suscepts were not thought to be related, but in 1929, Smith (11) described observations and experiments which indicated that the disease on narcissus was the same as that on amaryllis. Recent experiments by Feekes (4) gave similar indications.

Leaf scorch has been reported from many places in western Europe, and from the United States and Canada; its distribution is probably coextensive with that of narcissus and amaryllis culture.

The disease is responsible for considerable damage in large fields of narcissus grown for the flower and bulb market, since the leaves are killed 4 to 8 weeks before they normally die. The actual loss resultant from premature death of the leaves has not been determined experimentally, but growers estimate that the loss in weight of bulbs may be as much as 30 per cent or more. Since all food normally stored in the bulb is elaborated in the green leaves, this reduction is attributable to partial starvation caused by the premature death of the foliage. Ordinarily the disease on narcissus does not assume epiphytotic proportions until after the flowering period, so that the flowers usually escape infection. In southern regions, however, the buds and flowers also may be damaged.

It seems that no comprehensive study of leaf scorch has ever been made. Reports of preliminary studies have been published, but even in these the general characteristics of the disease and its causal organism are inadequately described. It is the purpose of this paper to present results of observations and experiments as a contribution to a more complete understanding of the disease and the pathogen as it occurs on narcissus in the eastern United States.

SYMPTOMS

The initial symptoms of narcissus leaf scorch appear very early in the season as yellowish, reddish, or brownish lesions at the tips of the first few leaves (Fig. 2, A). These lesions may be evident as soon as the tips have reached the surface of the soil, or they may not appear for some time thereafter. Typically the lesion is confined to the upper 2 or 3 inches of the leaf (Fig. 2, B). The older parts of the lesion soon become necrotic and often zonate in appearance. The transitional area between healthy and

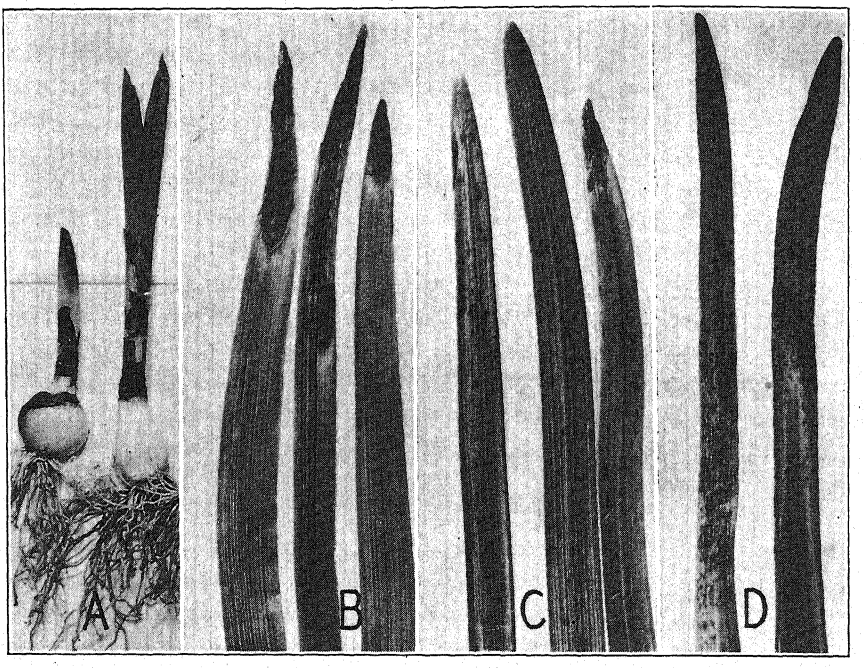


FIG. 2. Symptoms of narcissus leaf scorch. A. Primary lesions at the tips of very young leaves and sterile sheaths (horizontal line represents soil surface). B. Primary lesions at tips of older foliage leaves. C. Secondary lesions initiated by inoculum from primary lesions, leaf to the right bearing primary lesion at tip and secondary lesions below. D. Appearance of secondary lesions after leaves have been killed by the disease, showing the dark reddish brown scab-like spots as they appear against the lighter grayish brown tissue surrounding them.

diseased tissue is typically yellow. Since these lesions at the tips of the leaves are the first to appear, they may be called primary lesions, while those brought about by inoculum from primary lesions may be called secondary lesions.

Secondary lesions first appear as minute water-soaked spots characterized by yellow centers, as they age. Soon the centers become reddish brown and necrotic as the area of the lesions expands (Fig. 2, C). Secondary lesions rarely measure over $\frac{1}{8}$ in. $\times \frac{1}{2}$ in. and usually remain much smaller. The spots may be so numerous and close together as to coalesce. Eventually the tissue surrounding these spots dies and becomes light grayish brown. Distributed in this dead tissue, the spots appear as somewhat raised, scabby, dark reddish brown areas (Fig. 2, D). These secondary lesions cause the greater part of the damage because of their frequency and common occurrence on the lower portions of the leaves. The leaves die very quickly after the appearance of secondary lesions.

Numerous dark pycnidia, which serve as signs of the disease, project through the surface of the necrotic tissue. They are abundantly produced, usually in both primary and secondary lesions proper, as well as in the light grayish brown, dead tissue surrounding the lesions.

Since the symptoms of leaf scorch and those of another common disease known as "fire," caused by *Botrytis* spp., are somewhat similar in that both cause premature death of leaves, they may be confused. Moreover, both may occur in the same planting and even on the same leaf. It is believed, however, that they may be distinguished with some degree of accuracy by the presence of the reddish brown, scab-like spots in the dead portions of infected leaves in the case of leaf scorch, and by the absence of the scab-like spots in the case of fire. The presence of pycnidia in the necrotic areas in leaf scorch and the fuzzy grayish masses of conidiophores in Botrytis fire are also of diagnostic value.

THE PATHOGEN

Name and Classification. Leaf scorch is caused by the fungus *Stagonospora curtisii* (Berk.) Sacc. It appears that the fungus was first collected by Curtis, at Society Hill, South Carolina. It was named by Berkeley and, in 1878, described by Cooke (2). Berkeley gave it the name of *Hendersonia curtisii.* The description given by Cooke is as follows:

Hendersonia curtisii Berk. in herb. Curt. Sporis elongato-ellipsoides demum biseptatis, 0.017–0.021 by 0.0075 mm. Ad narcissum.

Berkeley, also, established the genus Hendersonia, and in the same paper Cooke gives the following description:

Hendersonia Berk. Ann. Nat. Hist. Perithecia carbonacea, sporis elongatis, bi vel multiseptatis, hyalinis vel coloratis, primo pedicellatis demum liberis (Sporocladus corda proparte).

In 1887, Saccardo (9) separated Berkeley's genus Hendersonia into two groups of generic rank on the basis of spore color. The species with hyaline spores constituted the new genus Stagonospora, while those with colored spores were retained in Hendersonia. Saccardo, therefore, lists the leaf-scorch fungus as *Stagonospora curtisii* (Berk.) Sacc.

A very scant specimen collected at Society Hill, South Carolina, by Curtis is in the Curtis herbarium at Harvard University. The collection is so meager that proper study and comparisons could not be made. Sketches of spores on the specimen packet indicate that they consist of 1 to 3 cells and resemble the spores isolated from narcissus leaves affected with scorch. Since no note accompanied these sketches, it could not be determined by whom they were made. These sketches and the brief description by Cooke indicate that *Hendersonia curtisii* Berk. is the same organism that has proved to be the cause of the leaf-scorch disease.

In 1900 Aderhold (1) isolated a fungus from narcissus leaves in Germany, which he named *Phyllosticta narcissi,* n. sp. Upon the same leaves he also found spores of a fungus resembling those of *Stagonospora curtisii.* Since it is now known that the spores of the leaf-scorch fungus are so variable, indications are that Aderhold did not have 2 distinct fungi, but one and the same organism. The description of the diseased leaves from which he isolated *P. narcissi* also indicates that he was dealing with leaf scorch.

In recent studies Smith (11) and Feekes (4) recognized the organism as *Phyllosticta narcissi* Aderh. but suggested the possibility of its being the same as *Stagonospora curtisii.*

A comparative study of the descriptions of *Phyllosticta oudemansii* by Saccardo (10) and *Stagonospora narcissi* by Hollos (5) occurring on narcissus, and *Phyllosticta* sp. by Dougherty (3), *P. gemmipara* by Zondag (12), *Phoma amaryllidis* by Kotthoff and Friedrichs (7), and *Stagonospora* sp. by Petrak (8), occurring on amaryllis, has led the writer to believe all are identical and are like the narcissus leaf-scorch fungus.

These differences in opinion concerning the identity of the pathogen very probably have been due to its occurrence on two different hosts and the variability in size and spore septation. Because of priority the proper name of the organism is, therefore, *Stagonospora curtisii* (Berk.) Sacc.

Pathogenicity. The pathogen was isolated from leaves, buds, flowers, and bulb scales. Isolations were made from the small reddish brown spots in green leaves, as well as from dead leaves bearing pycnidia. The organism was isolated also from the leaf sheaths near the soil surface, as well as from the outer scales of bulbs.

The pathogenicity of *Stagonospora curtisii* has been established by inoculating narcissus leaves with mycelium and spores. A number of freshly wounded and sound leaves of the variety Sir Watkin, growing in the greenhouse, were inoculated with mycelium from cultures on potato-dextrose agar. In several cases a piece of agar containing the mycelium was placed near the bases of the older leaves so that such young leaves as emerged subsequently might come in contact with the fungus. Leaves of the check plants were wounded or were left unwounded, and sterilized agar was placed in the wounds and on the uninjured parts. Bell jars were placed over all of the plants to maintain a high humidity.

Within 24 hours the first symptoms of disease became evident as water-soaked areas around the wounds. In 48 hours the centers of the lesions had become necrotic and brown and were surrounded by a yellowish area almost an inch in length. At the same time yellow areas appeared on the side opposite the inoculum on the sound leaves. In 3 days numerous pycnidia appeared in the dead areas surrounding the wounds and the

lesions extended much farther. The lesions of the sound inoculated leaves had also enlarged and were necrotic in the centers. After a week the bases of the older leaves and the tips of younger leaves that came in contact with the blocks of agar containing the organism exhibited typical leaf-scorch lesions. Twenty days after inoculation the leaf tips on the inoculated plants were dead and dry, while those of the check plants were perfectly healthy (Fig. 3, A). A repetition of these experiments in the greenhouse and in the field gave similar results.

A suspension of spores in distilled water was atomized on leaves of a variety of *Narcissus poeticus* growing in the field. A large moist chamber was placed over the inoculated plants and another was placed over plants near-by to serve as a check. The chambers were removed after 48 hours. In 4 days numerous reddish brown spots were faintly apparent on the leaves sprayed with spores, while the check plants were entirely free from spots. After 10 days the inoculated leaves exhibited the typical spots resembling the secondary lesions that occur naturally in the field and greenhouse. Four weeks after inoculation nearly every leaf in the spore-sprayed plot was dead, shriveled, and dry, while the leaves in the check plot were free from infection (Fig. 3, B and C). This experiment, when duplicated, yielded similar results.

A comparative study of the pathogenicity of the 2 organisms isolated from amaryllis and narcissus, respectively, has been made. The fungus from both sources produced the typical red spots on the leaves of amaryllis and the typical leaf-scorch symptoms on narcissus. These results and observations verify the conclusions of Smith (11) and Feekes (4), *i.e.*, that one and the same organism is responsible for the disease on the 2 suscepts.

Life history. All evidence available suggests that the fungus overwinters in or on the bulbs, and that this is the principal source of inoculum for the primary cycle. The organism has not been isolated from bulbs a sufficient number of times to establish this point beyond a doubt, but the following observations and results of experiments tend to support this probability.

Ninety narcissus bulbs of the variety Queen of the North, whose leaves were destroyed prematurely by the leaf-scorch pathogen during the previous season, were taken from Long Island, New York, to Durham, North Carolina. They were planted in soil in which narcissus had never been grown. The next season over 100 leaves bore primary lesions. This result confirms observations of experienced growers and indicates that the pathogen is carried on the bulbs. In another experiment, 90 bulbs of the variety Laurens Koster were used, the leaves of which had also been destroyed prematurely by the leaf-scorch pathogen during the previous season. One-

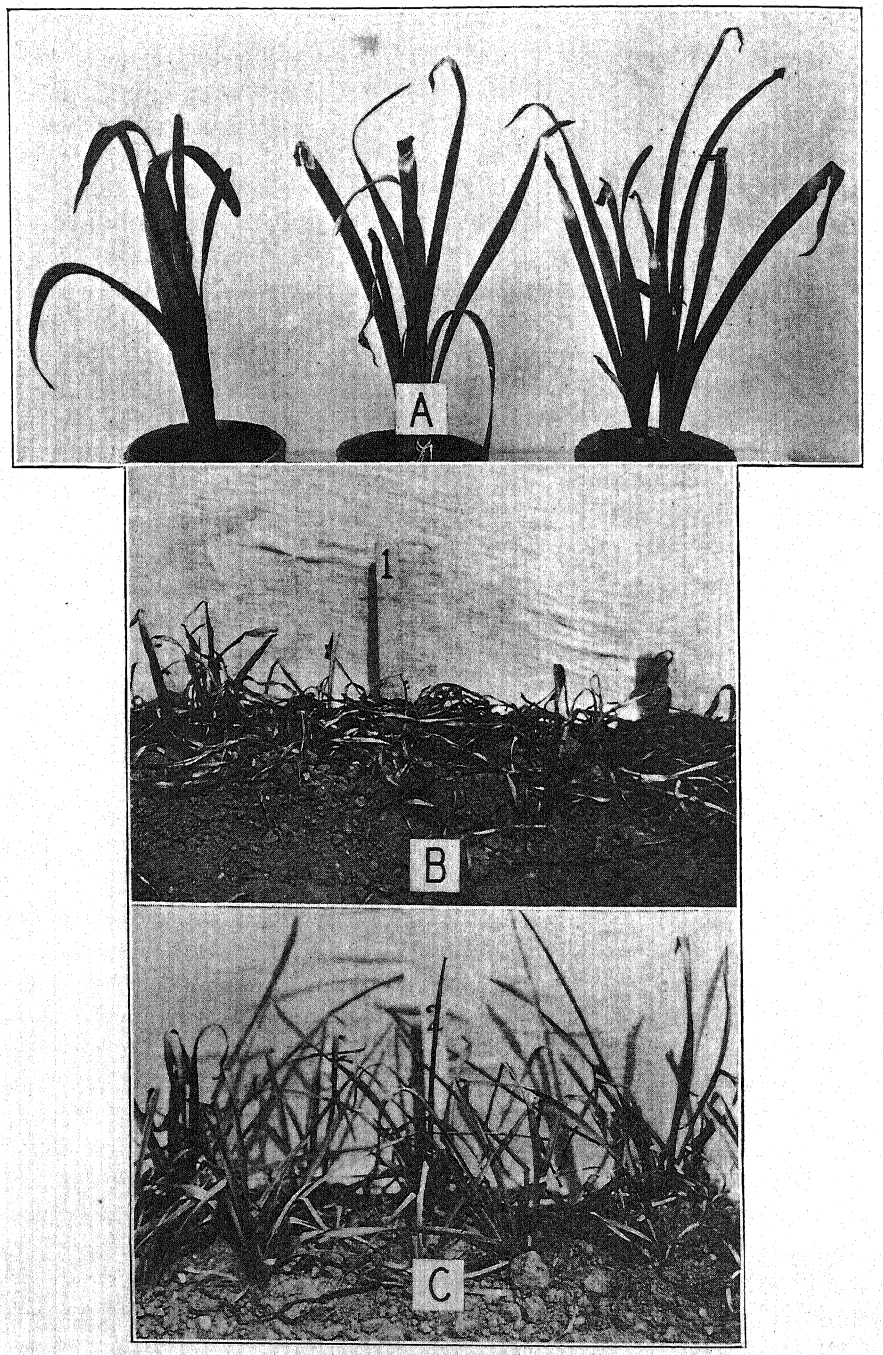


FIG. 3. Results of inoculation experiments. A. Narcissus leaves killed at the tips as a result of inoculation with mycelium. Check plant at the left. B. Result of inoculation with spore suspension, photographed four weeks after inoculation. C. Plants used as a check against those inoculated in B, leaves beginning to die naturally because of old age but entirely free from leaf scorch.

half of this number were treated for 2 hours in one-half per cent Formalin solution immediately before planting, while the remaining 45 bulbs were not treated. Each lot was planted in a row about 2 feet from each other in soil in which narcissus had never been grown. The following season 18 leaves of the nontreated lot exhibited typical primary lesions and by the end of May 62 leaves were infected, while not a single primary lesion appeared in the lot treated with Formalin. These results indicate that the fungus is on or in the tissue of the bulb scales, since infection occurred in leaves of the nontreated lot and not in those treated with Formalin.

It has not, however, been definitely proved that the fungus overwinters on the bulbs nor are the form and exact location of the fungus during the dormant period definitely known. This is especially true of bulbs grown in accordance with present commercial practices, but, under rather unusual conditions, pycnidia and mycelium of the pathogen were found growing in the outer scales in the neck region of 2 bulbs. These bulbs, with necks and upper halves exposed, were growing in an old naturalized planting in an orchard near Norfolk, Virginia. In another instance the dead scales in the neck region of a bulb were broken off and had been carried upward in the form of a ring about the growing leaves. At the point of contact with the ring of dead tissue a primary lesion appeared on

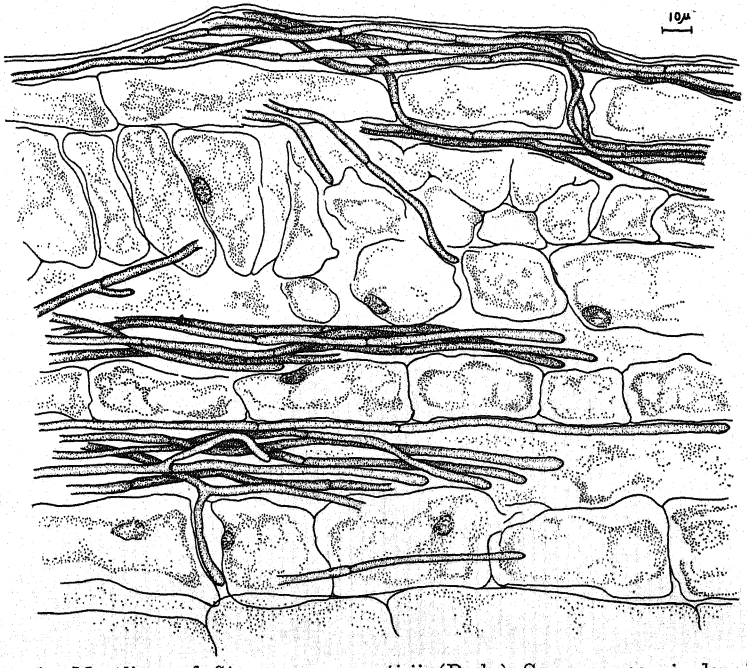


FIG. 4. Mycelium of *Stagonospora curtisii* (Berk.) Sacc. as seen in longitudinal section of narcissus leaf.

each leaf. Upon examining the dead scales, numerous pycnidia and mycelium of the leaf-scorch fungus were found.

According to Feekes (4), the fungus has been found as a loose, grayish, mycelial web over the outer surface and between the scales of dormant bulbs. He suggests that the fungus may survive the winter in this form. Thus far, the writer has never observed the mycelial web on any bulbs he has examined.

After the dormant period, the new leaves grow out of the bulb and come in contact with the dead basal portions of the previous season's foliage leaves in the neck region. It is believed that the tips of the new leaves are inoculated with the pathogen as they pass this region, since lesions are usually well advanced when the tips come through the surface of the soil, and only a few of the outer leaves bear primary lesions.

The primary lesions contain numerous intercellular hyphae of the pathogen, which not only grow between the cells of the mesophyll, but even penetrate between the epidermis and cuticle (Fig. 4). Commonly, a rather

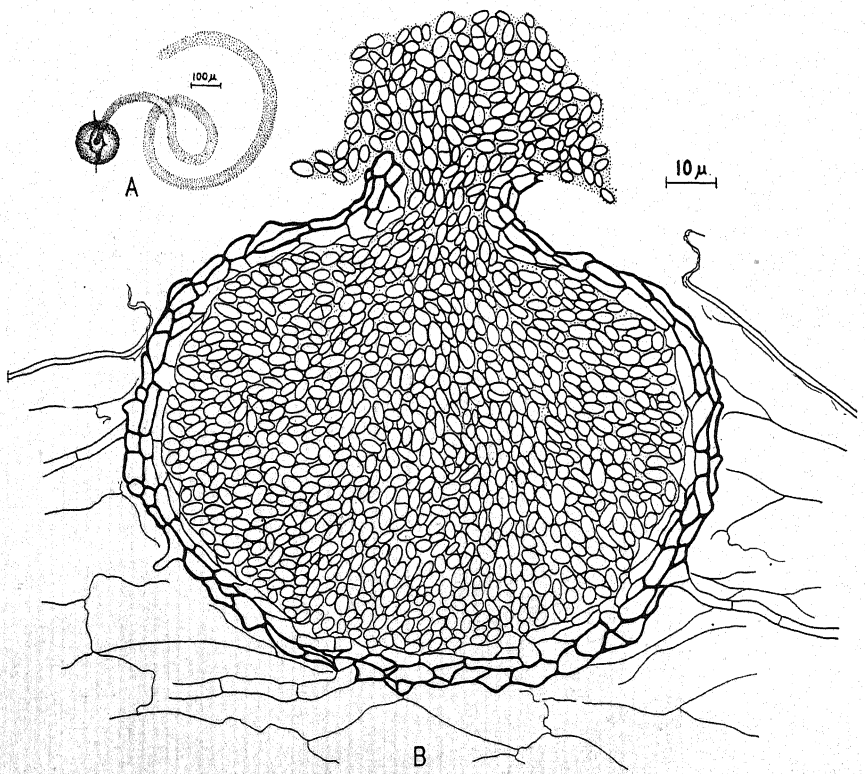


FIG. 5. Pycnidia of *Stagonospora curtisii* (Berk.) Sacc. A. Pycnidium as seen from above, showing the gelatinous matrix and spores oozing out in a cirrus. B. Median vertical section through a mature pycnidium produced in a living leaf in the greenhouse.

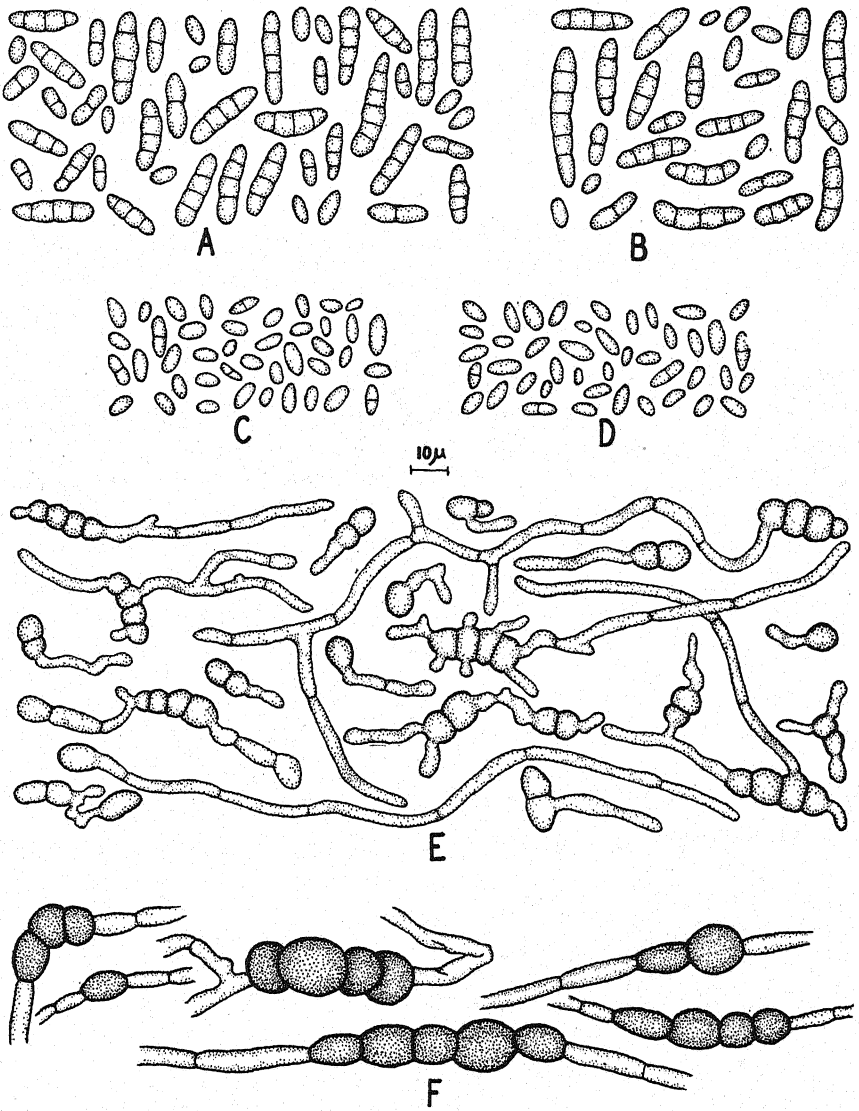


FIG. 6. Spores of *Stagonospora curtisii* (Berk.) Sacc. A. Formed in necrotic areas of leaves in the field following natural inoculation. B. Produced by an isolation from narcissus, in living leaves of narcissus in the field as a result of artificial inoculation. C. Produced by an isolation from narcissus in autoclaved narcissus leaves. D. Produced by an isolation from amaryllis in autoclaved narcissus leaves. E. Germinating spores. F. Thick-wall resting cells or chlamydospores.

distinct hyphal mat develops between the thick cuticle and the outer cell walls of the epidermis. In living tissue the progress of mycelial growth is relatively slow, but after the tissue dies growth is very rapid.

Pycnidia soon develop subepidermally in the dead tissue. They appear as minute brown or black, papillate, globose bodies. Each pycnidium opens by an ostiole at the leaf's surface through which multitudes of spores ooze out in cirrus formation. The spores are embedded in a gelatinous matrix and when the pycnidium becomes wet, water is imbibed by the gelatinous substance, causing it to swell greatly. As a result the matrix oozes out through the ostiole carrying with it all of the spores (Fig. 5). The principal agent of spore dissemination is water during rains. Splashing rain carries spores from the infected areas to healthy areas of the same leaf and to other leaves of the same and near-by plants. Wind may aid in disseminating spores during rains by carrying spore-laden droplets. Spores produced in the primary lesions constitute the inoculum that initiates the principal secondary cycles of infection.

The spores germinate in water within several hours (Fig. 6, E). The first obvious change is a marked swelling of each cell and, at the same time, the walls may become slightly olive brown. One to several septate germ

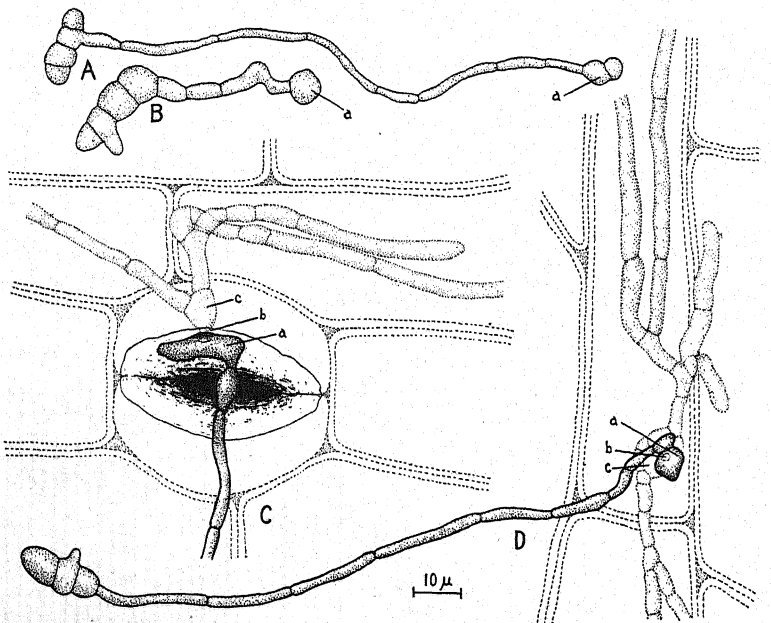


FIG. 7. Entrance of the pathogen into the tissue of the leaf. A and B. Appressoria (a) formed at the tips of germ tubes. C. Young mycelium invading the subcuticular region of the leaf, showing the appressorium (a) which formed against one of the guard cells of a stoma, the small invasion-hypha (b), which grew from the underside of the appressorium and penetrated the thick cuticle. D. Mycelium developing between cuticle and epidermis of a leaf, showing the appressorium (a) at the tip of the germ tube, the small invasion hypha (b), seen in vertical section, and the typical vesicle (c) from which the intercellular hyphae originated.

tubes develop from one cell, or from all cells of a spore. The germ tubes grow over the surface of the leaf and develop appressoria at their tips (Fig. 7, A and B). Soon a small invasion hypha develops from the underside of the flattened appressorium and penetrates the heavy cuticle. After this hypha grows through the cuticle and reaches the outer wall of the epidermal cells a more or less enlarged vesicle forms, which is sometimes composed of several cells. One to several hyphae grow from the vesicle and rapidly develop into an intercellular mycelium (Fig. 7, C and D). The present of the mycelium causes infection and a consequent secondary lesion.

Secondary cycles of infection also may be initiated by actual contact of diseased leaves and flower parts that bear primary lesions with healthy parts during damp and rainy weather.

Secondary lesions are formed abundantly over all parts of the leaves and are especially numerous near the base. The mycelium in the leaves grows downward toward the neck region of the bulb, as the leaves gradually die, and very probably remains alive in the remnants of dead leaves during the dormant period of the bulb. It has been demonstrated that a desiccated culture of the fungus can remain alive more than a year and that transfers will grow vigorously on fresh bulb agar.

Morphology. The intercellular mycelium in the succept tissue is composed of septate hyphae the branches of which usually originate immediately below the septa (Fig. 4). The hyphae in the margins of the lesions may be composed of relatively long cells with no apparent constrictions at the septa. In the older portions, however, they may be composed of relatively short and rounded cells with very prominent septal constrictions. The young hyphae are colorless and the older portions gradually become dark brown. The older hyphae, in moist tissue, may remain colorless or faintly colored for a relatively long time, but finally become darker.

The mycelium grows well on a number of agar media including potato dextrose, and extracts of bulbs of narcissus, tulip, iris, and onion, and of narcissus leaves. It has also been grown on autoclaved leaves of narcissus and amaryllis. The variations of mycelial growth on the different media are not marked. Since a very luxuriant growth developed on narcissus bulb agar, resembling that on autoclaved narcissus leaves, the characteristics as exhibited on that medium, only, will be described. When very small pieces of the mycelium were planted in Petri dishes maintained at room temperature (20 to 24° C.), mats of mycelium 3 to 5 cm. in diameter developed in 4 days. The mycelial mat was composed of both aerial and submerged hyphae. Aerial hyphae in mass are always some shade of gray, with usually an olive-green or olive-brown tint, and, with age, become dark brownish gray. The submerged hyphae in mass are dark olive brown

to black. The medium also becomes brownish to black, but is not so deeply colored as the submerged hyphae.

In autoclaved leaves, potato-dextrose agar, and narcissus-bulb-extract agar, thick-wall, dark, chlamydospore-like cells form abundantly in the older cultures. One to several adjacent cells, either terminal or intercalary, become enlarged, thick-wall and darker brown (Fig. 6, F). They serve as resting cells, as evidenced by the fact that under favorable conditions they give rise to new hyphae. Sometimes, by repeated division of these cells, sclerotium-like bodies are developed. It is believed that in some cases these same bodies function in pycnidial formation.

Pycnidia are globose to appressed-globose and ostiolate (Figs. 5, B, and 8, L). When crowded, or when for some other reason unequal pressure is exerted on their sides, they may be somewhat irregular. The mature bodies are 110 to 275 microns in diameter, the average being approximately 175 microns. The wall is composed of irregular, compressed, brownish cells, which form a layer 2 to 4 cells thick. The inner layer of cells is colorless, or nearly so, and the cell walls are much thinner than those toward the outside. The thin-wall cells that line the bottom and sides of the pycnidium project into the cavity and constitute the sporophores (Fig. 5, B).

The origin and development of pycnidia have been studied from growth on narcissus-bulb-extract agar and autoclaved leaves in Petri dishes. The best procedure for tracing pycnidium formation consisted in utilizing strips of epidermis attached to slides by means of the natural mucilage in the leaf tissue. These slides were placed in Petri dishes lined with several layers of water-soaked filter paper, which were then sterilized in the autoclave. A bit of mycelium was placed, aseptically, at one end of the epidermal strip. Within three days all stages of development from first indications of primordia to mature sporulating pycnidia could be observed in these epidermal strips. Cotton Blue in phenol and glycerine solution, when used as an *in toto* stain, was helpful in studying the early stages of pycnidial formation.

The origin and early development of pycnidia are rather variable (Fig. 8). Most commonly the primordium arises from a few adjacent cells of a main hypha. Several adjacent cells enlarge and repeatedly divide in all planes (Fig. 8, A–C). In the early stages they may appear identical with the chlamydospore-like cells previously described. Commonly, hyphal branches grow from the outer cells of the mass (Fig. 8, B–D). Some of these hyphae seem to take part in the development of the fruit-body, since they may branch and anastomose with the central mass of the primordium. Other branches, originating from cells of the main hypha some distance away from the developing pycnidium, may also anastomose with it (Fig.

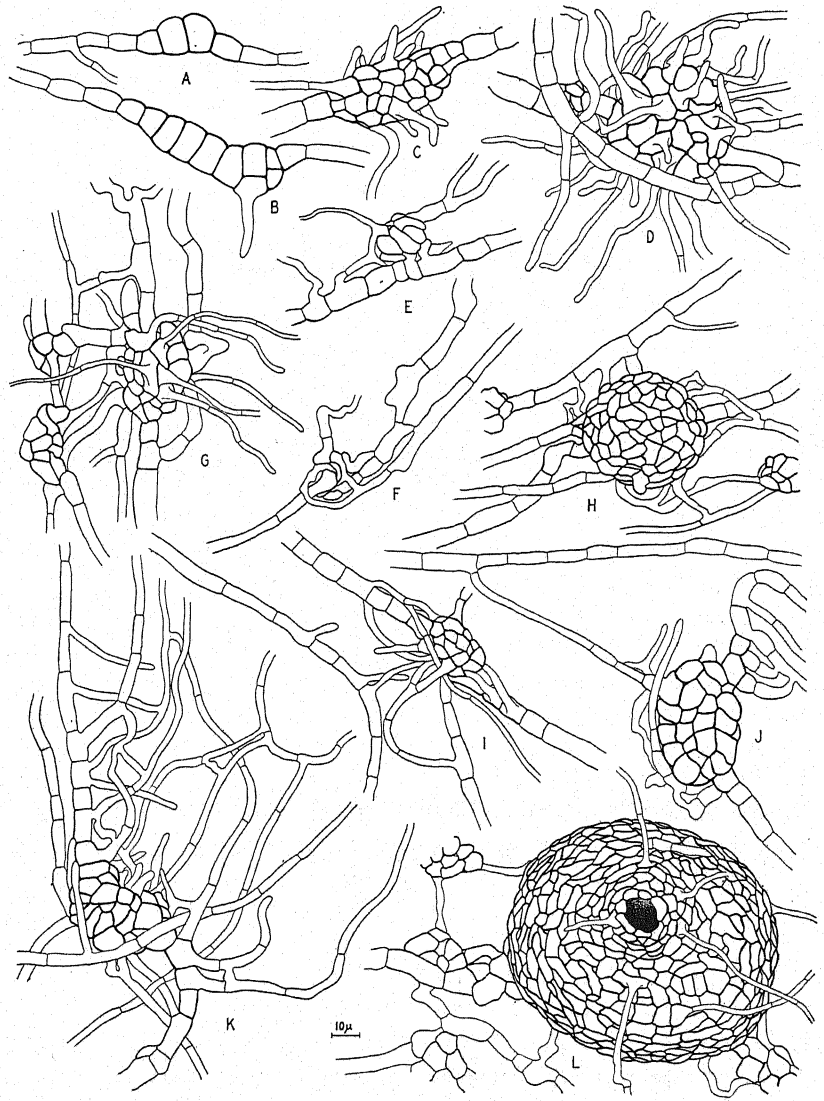


FIG. 8. Origin and development of pycnidia. A. Very early stage of pycnidial development. B. Slightly later stage. C. Multicellular pseudoparenchymatous stage with branches protruding from the mass. D. Later stage with branches protruding, some of which are rebranching, turning back and anastomosing with the primordium. E. Primordium formed by entwining and anastomosing of short branches at the side of a main hypha. F. Several branches entwining and anastomosing to form a primordium. G. Primordium in which several hyphae are involved. H. Similar condition in which branches from another main hypha have anastomosed with the primordium. I. Branches from the original hypha and from another main hypha near-by, anastomosing with the primordium. J. Similar condition, showing curious anastomoses of hyphal branches with the primordium, with one another, and with another hypha near-by. K. Hyphal branches from the primordium and cells of the original hypha near-by that have rebranched and anastomosed with the primordium and with one another to form an intricate network. L. Nearly mature pycnidium.

8, I). Hyphal branches from the primordium frequently anastomose with one another and other hyphae near-by (Fig. 8, G–K). The significance of anastomoses of these branches with one another and with other hyphae some distance away from the primordium proper can not be definitely explained, but they may perhaps aid in transportation of food from other hyphae to the developing pycnidium. Hyphal branches from main hyphae near-by have also been observed to branch and anastomose with cells of the primordium (Fig. 8, G–I). It is difficult to determine with certainty whether branches from other hyphae have anastomosed with the primordium, or whether they have grown from the primordium and fused with the near-by hyphae. There is some evidence that both conditions occur.

Another type of origin different from that described above has been observed in which short branches from the main hypha entwine and anastomose with one another, the primordium being formed at the side of the main hypha (Fig. 8, E). This method of origin is infrequent.

There is some indication that still another type exists in which several main hyphae branch, entwine, and anastomose with one another and gradually develop into the primordium by growth and multiplication of cells (Fig. 8, F).

According to Kempton (6), pycnidia originate and develop by 2 main methods, meristogenous and symphogenous. The meristogenous method resolves itself into 2 modes, simple and compound. In the simple mode the pycnidium develops from a single cell or a few adjacent cells of a single hypha; in the compound mode, adjacent cells of two or more continuous hyphae divide, swell, and sometimes branch, all of which then anastomose freely to form a pseudoparenchymatous mass. The type of origin of pycnidia of the narcissus leaf-scorch fungus is evidently a modification of the simple and compound meristogenous method, varying from one extreme to the other. This variable characteristic in pycnidial development is not uncommon among the members of the Sphaeropsidales.

Numerous spores are formed on the short sporophores and soon completely fill the cavity of the mature pycnidium (Fig. 5). They are exceedingly variable in size and septation, whether taken from an individual pycnidium or from pycnidia that have grown under different conditions (Fig. 6, A–C). Successive portions of mycelium from a culture were planted on narcissus-bulb agar, another on autoclaved narcissus leaves, another on living leaves of plants in the greenhouse under warm humid condition, and another on leaves in the field under normal conditions. Spores produced on bulb agar, autoclaved leaves, and in living leaves in the greenhouse were very abundant, but all were very small, and nearly all were unicellular, with bicellular ones occurring rarely (Fig. 6, C). The

spores taken from leaves in the field were much larger and the majority were quadricellular (Fig. 6, B). Bicellular spores also were present, while unicellular ones were relatively rare. The spores produced by artificial inoculations in the field were similar to those produced in lesions formed as a result of natural infections (Fig. 6, A and B). Examination of various collections from the field shows that all these variations of spores may occur. Smith (11) also has recognized the extreme variability of the spores of this fungus.

The spores are hyaline, elliptical to fusiform to nearly cylindrical, bluntly rounded at both ends, consisting of 1 to 6 cells, usually slightly constricted at the septa; spores of 1, 2, and 4 cells are common; those with 3, 5, and 6 cells, rare; size variable; unicellular spores, 4.5 to 10.2 by 3.2 to 4.8 microns, average 7.5 by 3.8 microns; bicellular spores 8.0 to 16.0 by 3.2 to 6.4 microns, average 11.5 by 4.5 microns; quadricellular spores 13.4 to 28.0 by 4.8 to 8.0 microns, average 21.0 by 6.5 microns.

SUMMARY

This investigation deals with leaf scorch of narcissus, a disease that kills part or all of the leaves of susceptible varieties 4 to 8 weeks before they should normally die. The disease also occurs on amaryllis.

The initial symptoms of the disease in narcissus appear as yellowish, reddish, or brown lesions that soon develop necrotic areas at the tips of the first leaves. Later in the season numerous secondary lesions appear as reddish brown spots on leaves near the primary lesions. It is the secondary lesions that effect the greater damage.

The disease is caused by the fungus *Stagonospora curtissii* (Berk.) Sacc.

Pathogenicity has been proved by inoculation with mycelium and with spores.

Experimental results and observations indicate that the fungus overwinters in or on the bulbs and that this is the principal source of inoculum for the primary cycle of infection.

Numerous pycnidia are produced in necrotic areas of the lesions. Their origin is somewhat variable, arising by modification of the simple and compound meristogenous methods.

Spores are exceedingly variable in size and septation. Under some conditions nearly all spores are very small and unicellular with bicellular ones occurring rarely, while under other conditions the majority are quadricellular.

The principal agent of spore dissemination in the field is water during rains.

The fungus gains entrance directly through the cuticle after forming an appressorium at the tip of the germ tube. An intercellular mycelium is formed.

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OVERWINTERING OF PHYTOMONAS PRUNI ON PEACH

H. H. THORNBERRY[1] AND H. W. ANDERSON

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The overwintering of *Phytomonas pruni* on peach has been reported by Rolfs (6), Roberts (5), Adams (1), Higgins (4), and Dunegan (3) to occur in twig cankers. Rolfs (6) also mentioned that in some cases bacteria in infected bud tissue were the source of the spring inoculum. Anderson (2) presented evidence that the pathogen can survive the winter in diseased fallen leaves. Since he obtained negative results in his isolation from buds and from overwintering twig cankers he considered old fallen leaves as a possible source of inoculum in the spring. On March 22, 1930, a new type of twig canker was discovered at Olney, Illinois. This type will be referred to below as "spring canker" to distinguish it from the ordinary canker formed during the summer months.

DESCRIPTION OF SPRING CANKERS

The spring cankers, developing on young succulent twigs of the past summer's growth, form water-soaked, slightly darkened blisters about the time the first leaves appear. At first they are small, raised areas about 2 mm. in diameter, but later extend from 1 to 10 cm. parallel to the long axis of the twig (Fig. 1, A). They seldom extend more than one-half the circumference of the twig but may surround it. The bacteria are limited to the cortical tissue (Fig. 2, A). Occasionally, in cankers near the terminal bud, the infection surrounds the twig and a type of die-back results (Fig. 3). Later in the spring the epidermis over the lesion is ruptured (Fig. 4) and bacteria are exposed for dissemination by rain and dew to near-by leaves. After the epidermis is ruptured the infected tissue, darkening and drying during summer, results in deep cankers (Fig. 1, B), which persist through the following winter. Occasionally, several small separated blisters may coalesce to form a single large canker. They develop before green shoots are formed in the spring. Under the microscope the infected tissue is seen to be invaded intracellularly (Fig. 2, C) by abundance of bacteria. These ooze from the cut surface of sections mounted in water.

SUMMER CANKERS

The summer cankers on green shoots (Fig. 5) develop later in the summer, usually after foliage infection has become established. In 1932, for example, they did not appear until mid-August. At first they are water-

[1] Formerly assistant in Pomological Pathology, Ill. Agr. Exp. Sta. Now with Rockefeller Institute for Medical Research, Princeton, N. J.

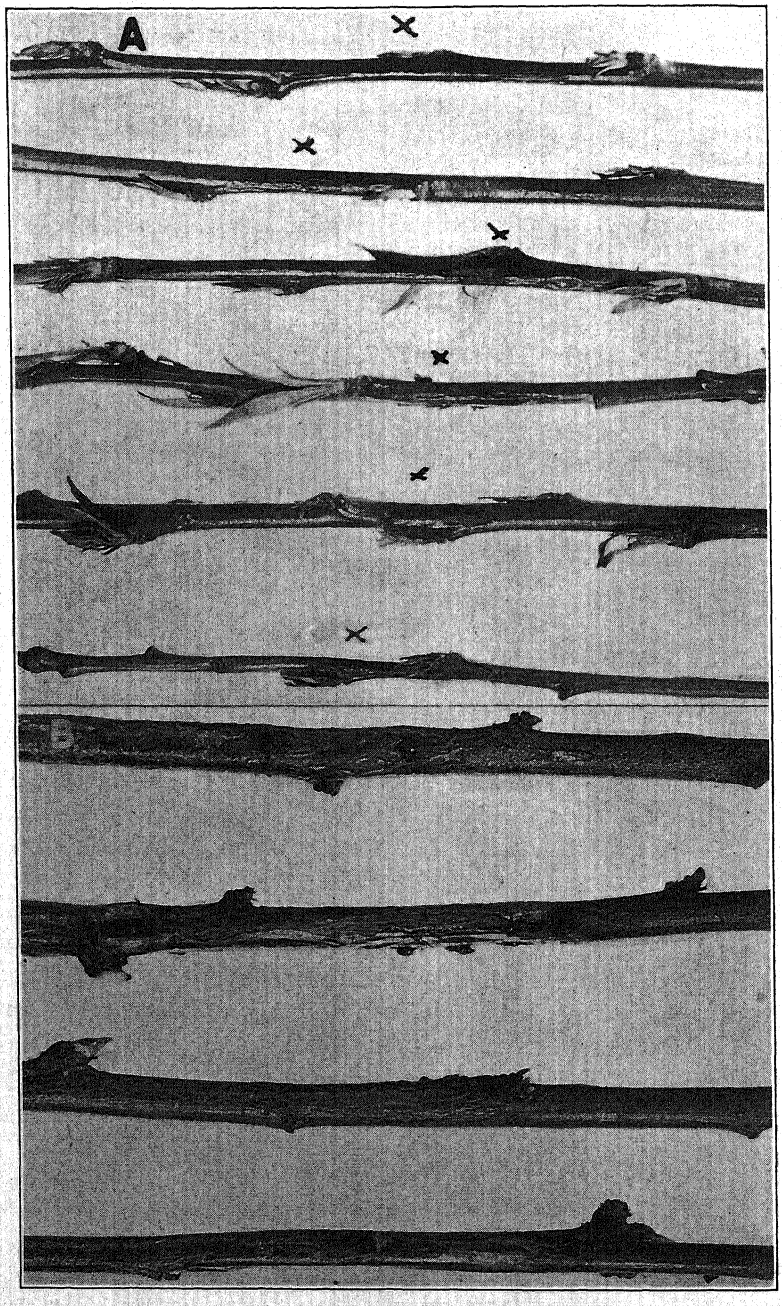


FIG. 1. Spring cankers on twigs and water sprouts. A. Young specimens collected at Villa Ridge on April 1, 1930. B. Note splitting and healing of overwintered specimens collected at Carbondale and Villa Ridge on May 20, 1930.

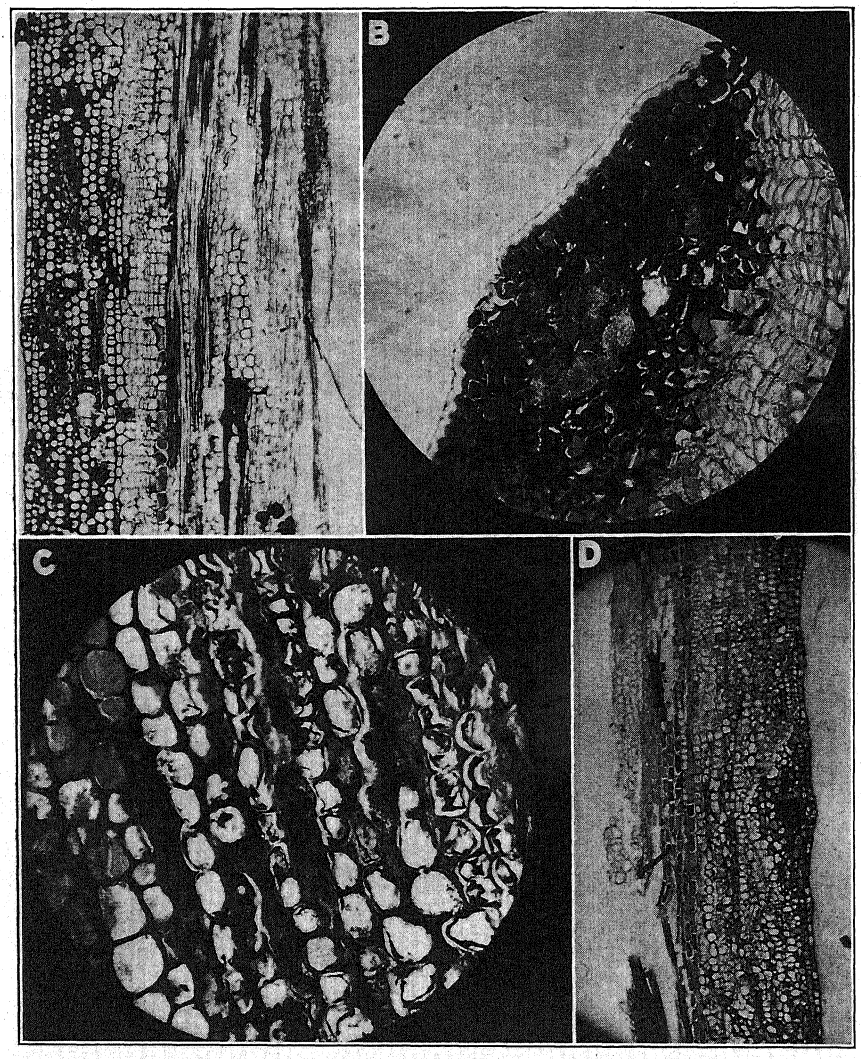


FIG. 2. Photomicrographs of stained sections. A. Masses of bacteria in the intercellular spaces of the cortical tissue of a young twig. Dark areas are bacteria. ×90. B. Bacteria in tissue. ×740. C. Same as A. ×740. D. Same as B. ×90.

soaked, dark purplish lesions that later dry out and show the characteristic sunken canker of darkened tissue.

PRIMARY FOLIAGE INFECTION

Natural, primary foliage infections in Illinois were correlated with the spring cankers during the early springs of 1930 and 1931 by the writers

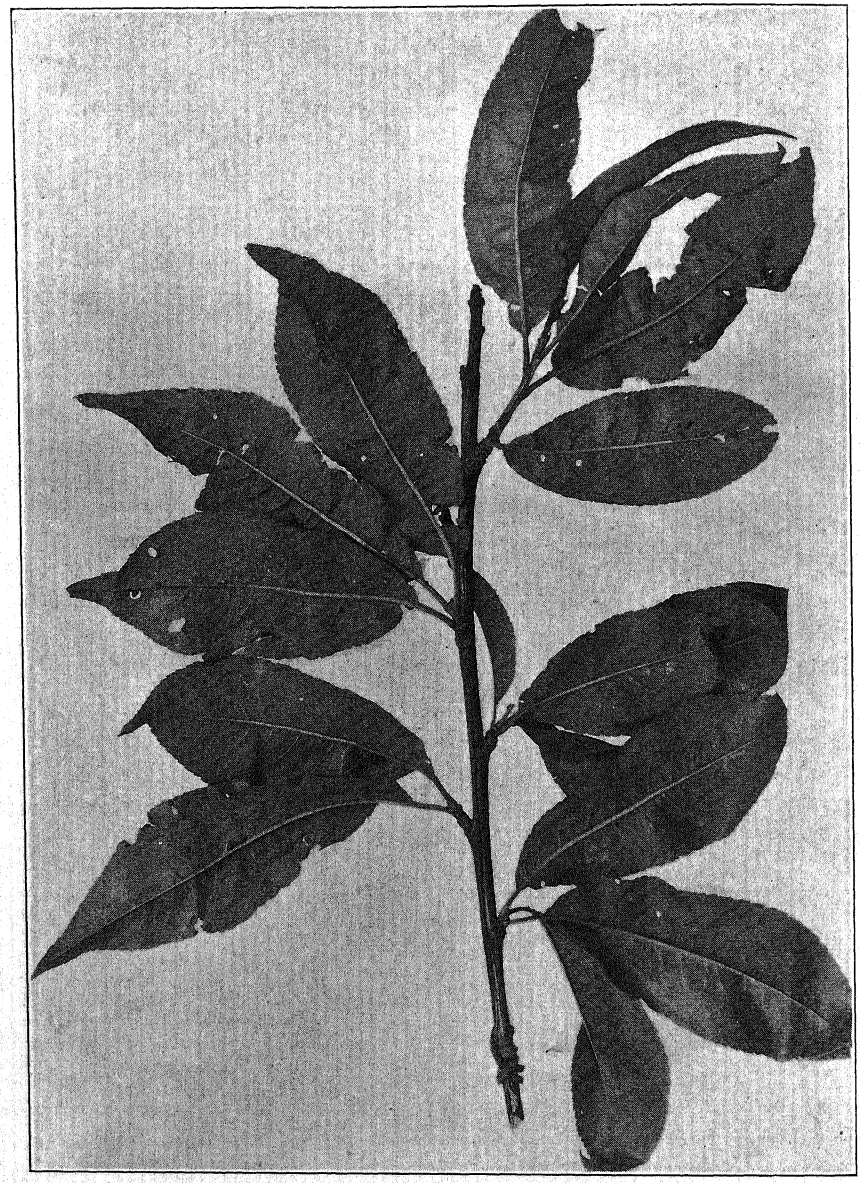


FIG. 3. Primary foliage infection near a spring canker that caused a die-back of the twig. Collected at Urbana on May 12, 1931.

and in 1932 by the junior writer. Due to the scarcity of cankers in general and also the difficulty in locating them, it was much easier to find the twig lesions after primary infection was evident. However, many cankers were found before there was any evidence of foliage infection.

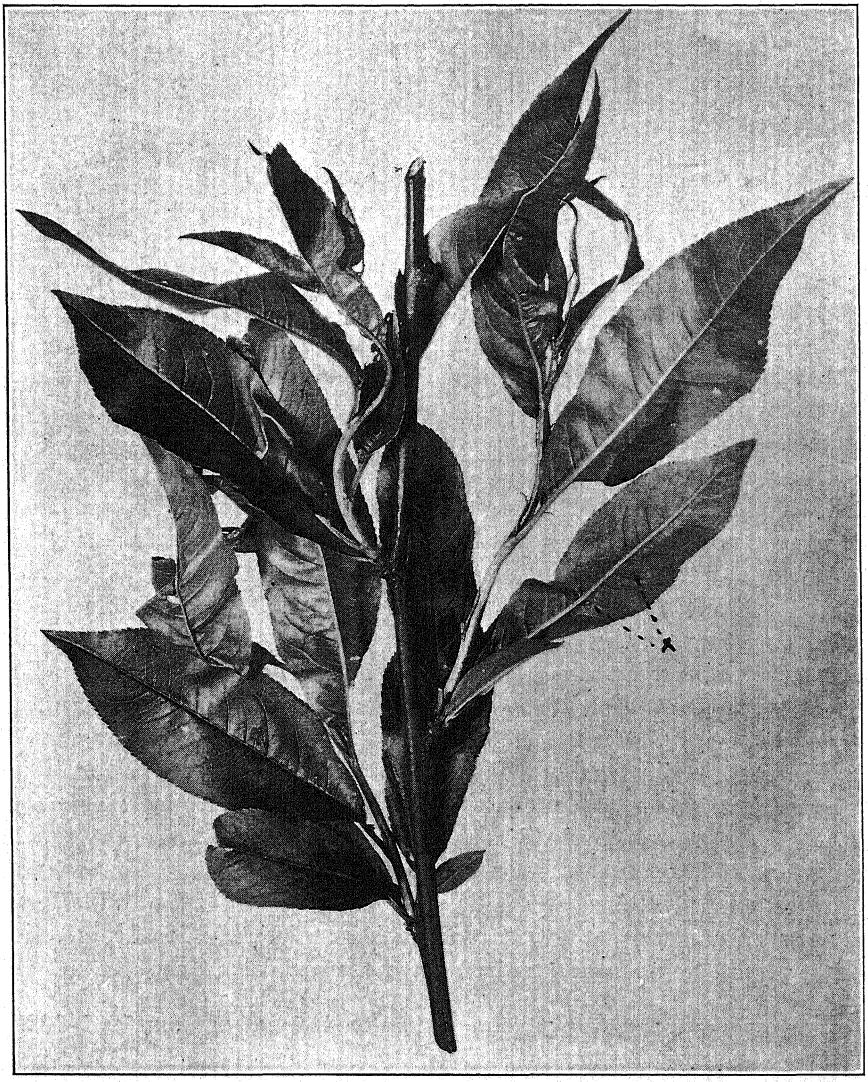


FIG. 4. Primary foliage infection, shown as dark areas on leaves, located below a spring canker at the base of the third shoot from the bottom. Canker has burst open. Collected at Villa Ridge on April 14, 1930.

In 1930, cankers were first observed in the University of Illinois peach orchard at Olney, Illinois, on March 22. At this time the early leaf buds had opened and no evidence of foliage infection was found. On April 1, at Carbondale, Villa Ridge, and at Irvington, cankers were found, but foliage infections were not evident near the cankers or elsewhere in the

orchard. On April 13, at Irvington, 7 leaves with very small spots that were quite typical of young lesions were found near a canker, while no infection could be found elsewhere in this orchard. Microscopical examination in the field showed bacteria oozing from the cut surface of one lesion. At Villa Ridge, on the 14th, light localized infections were found near cankers but none was noted in any other part of the trees. At Carbondale, however, on the same day, several cankers were observed but no foliage infection was found. At the time of the next observations, April 25, at Irvington and Carbondale, and April 26, at Villa Ridge and Olney, primary leaf infections were quite evident but only near cankers. The bacteria evidently had spread about one foot from the cankers. By the time of the next observations, May 20 and 21, the infection appearing throughout the trees prohibited further correlation of initial infection with spring cankers.

During the winter months of 1930 and 1931 the writers examined twigs at Urbana and in southern Illinois in order to determine when the cankers become evident. The study was carried out in orchards where the disease was prevalent the previous summer. On December 18 a careful search at Irvington, Carbondale, and Olney revealed no evidence of visible blistery cankers. However, the pathogen was isolated from a dead terminal bud collected at Olney. Again, at the same places on January 28, no cankers were found. On March 13 no cankers were observed either at Irvington or at Villa Ridge. On the 14th, at Carbondale, one small spring canker about 5 mm. in diameter was found on the south side of a water sprout. Isolation and microscopic examination the following day showed the pathogen throughout the lesion. On the 17th, at Carbondale, and 21st, at Irvington, a few cankers were observed without any foliage infection. However, on April 22, at Carbondale, 23, at Ozark, and 24, at Villa Ridge, primary infections were found near cankers. By May 10 the infections had appeared beyond the localized stage centered about cankers.

OBSERVATIONS ON PRIMARY INFECTION AT IRVINGTON, ILLINOIS, 1931–1932

An Elberta peach orchard near Irvington, Illinois, has yielded interesting results on overwintering, as it has always exhibited an abundance of bacterial spot. During the winter of 1931, a number of twigs showing typical summer cankers were collected and isolations attempted, with uniformly negative results.

On March 26, this orchard was examined for spring cankers but none could be found. Late in April primary infection was observed on a few leaves and, on May 24 and 25, a survey was made. From 85 trees, 70 typical spring cankers were obtained, mostly by examining the twigs near

cases of primary infection. Of these 70 cankers, 66 showed bacteria oozing from the cut outer bark when examined under the microscope, while 4 were negative. In addition, 53 tip-injured twigs that looked suspicious or were near primary infections were examined. Of these, 16 showed oozing bacteria, while 37 were negative. Those giving positive results were in most cases definite spring cankers at the base of the dead tip.

In addition to these observations, numerous summer cankers, formed in 1931, were examined for primary infection in the neighborhood and for oozing bacteria. The results were entirely negative.

EXPERIMENTS WITH PRUNED TREES

Further evidence that the source of primary inoculum may be traced to spring cankers was gathered at Urbana by a pruning experiment on 6 Brackett peach trees. These trees had had severe bacterial spot over a period of 4 years. Hundreds of summer cankers were marked during the season of 1931 for a study of the time of development of these cankers and the relation of time of development to possible survival of the bacteria. Plates were poured from a total of 221 summer cankers from this variety in January, February, and March, 1932. None of these yielded *Phytomonas pruni,* a fact that agrees with results of numerous other attempts.

The 6 Brackett trees were somewhat isolated from the remainder of the trees in the peach orchard and the closest susceptible variety was 6 rows distant.

Two trees were so pruned as to remove all 2- and some 3-year-old wood. All of the 1931 twigs were removed from the 2 trees, while the other 2 were left intact. It was reasoned that if primary infection appeared only on the nonpruned trees at the time of the first evidence of infection in the orchard, it also could be reasoned that the source of the inoculum was from year-old twigs. It was realized that secondary infections would soon appear on the pruned trees, but the time and place of primary infection, whether or not associated with spring cankers, would give a clue as to the place of overwintering of the bacteria.

Primary infection in the University peach orchards at Urbana was unusually late in the summer of 1932. The first observed infection was on a Hale tree on June 2. The nonpruned Brackett trees showed a light leaf infection a few days later, with no infection on the pruned trees. Since the pruned trees came into leaf a few days later than the nonpruned ones and it was thought this might be a factor in primary infection, 10 days were allowed to elapse before records were taken. On June 12, a number of infection areas were observed in the nonpruned trees where the leaves showed a few spots. In most instances the source of the inoculum was

evidently from typical spring cankers. Neither of the pruned trees that had 2 years' growth removed showed infection. One of the trees having one-year twigs removed also was free of infection. The second had one area where the leaves on a single small branch were infected. An examination of this branch revealed a canker immediately below the point where a twig had been removed.

For 3 weeks following no diseased leaves were found on the 3 trees, while extensive secondary infection occurred on the nonpruned trees and the infection area on the one pruned tree increased in extent. During the latter part of August, diseased leaves were found on all trees.

It would appear from this limited experiment that the primary inoculum comes from cankers on year-old wood, and that the spring cankers rather than the typical summer type of canker constitute the probable source.

ISOLATIONS FROM CANKERS

Isolations from the spring cankers soon after they were found showed abundance of typical colonies of *Phytomonas pruni* in the plates. From a total of 530 plates, 265 isolations duplicated, all contained the pathogen (Table 1).

Similar isolations from cankers throughout the following winter and spring indicated that there was a great reduction of the number of bacteria

TABLE 1.—*Summary of isolation results secured from various specimens of spring cankers of peach*

Collection		Isolation			
Date	Locality	Date	Specimens	No. Plates	Results
3/22/30	Olney	3/24/30	20	40	40+
3/22/30	Olney	3/27/30	15	30	30+
3/22/30	Olney	4/3/30	20	40	40+
4/1/30	Villa Ridge	4/3/30	10	20	20+
4/1/30	Carbondale	4/3/30	30	60	60+
4/1/30	Irvington	4/3/30	40	80	80+
4/1/30	Carbondale	4/7/30	10	20	20+
4/2/30	Olney	4/3/30	10	20	20+
4/25/30	Carbondale	4/27/30	30	60	60+
4/25/30	Irvington	4/27/30	20	40	40+
4/25/30	Villa Ridge	4/27/30	30	60	60+
6/17/30	Dixon, Ky.	6/20/30	10	20	20+
7/2/30	Urbana	7/2/30	10	20	20+
3/14/31	Carbondale	3/16/31	10	20	20+
			265	530	530+

in the tissue. From a total of 370 plates, 185 isolations duplicated, one showed typical colonies of the pathogen. The organism from a subculture of a colony proved to be pathogenic when inoculated by pricks with a needle into green Elberta shoots on June 6, 1931 (Table 2).

TABLE 2.—*Summary of isolation results from overwintering summer cankers*

Collection		Isolation			
Date	Locality	Date	Specimens	No. Plates	Results
12/19/31	Irvington	12/20/31	15	30	30 –
1/28/31	Irvington	1/30/31	20	40	40 –
1/28/31	Carbondale	1/30/31	20	40	40 –
1/28/31	Tunnel Hill	1/30/31	25	50	50 –
1/28/31	Irvington	2/4/31	10	20	20 –
1/28/31	Carbondale	2/4/31	20	40	40 –
1/28/31	Tunnel Hill	2/4/31	10	20	20 –
3/14/31	Carbondale	3/16/31	5	10	1 +
3/14/31	Villa Ridge	3/16/31	10	20	20 –
3/14/31	Irvington	3/16/31	15	30	30 –
3/14/31	Carbondale	3/20/31	10	20	20 –
3/14/31	Irvington	3/20/31	10	20	20 –
3/14/31	Villa Ridge	3/20/31	15	30	30 –
			185	370	1 +

Diseased tissue from the cankers that develop on green shoots from natural infection during spring and summer (Fig. 5) also was used for plating. The cankers were located and labeled on July 10, 1929, at a time when their macroscopic identification was reasonably certain. Isolation from 5 on that date proved that bacteria were present in abundance. On January 5, 18, 24, 29, February 7, 13, 25, and 28, 1930, all the isolations were negative. Similar studies on the same dates with cankers produced by artificial inoculation at Urbana likewise developed no typical colonies of the pathogen. The inoculations with culture No. 66 were made into young green Elberta shoots by pricking with a needle on July 15, 1930. Typical lesions containing abundance of bacteria were evident July 25. These results agree with those of the junior writer, extending over a period of 10 years and involving approximately 500 attempts at isolation from summer cankers during the winter months. In not a single case was *P. pruni* isolated from such cankers after December 1.

OVERWINTERING OF THE PATHOGEN IN CULTURE

The pathogen in culture, protected from direct sunlight and rain, survived the outdoor winter conditions of 1930–31 at Urbana. Dextrose-beef-

extract agar slants, freshly inoculated with a culture of *Phytomonas pruni* were repeatedly exposed about the 1st and 15th of each month from November to March, inclusive. Transfers from the cultures to sterile slants on April 1 showed viable bacteria in all on April 5. There was noticeable growth in the exposed tubes during periods when the mean maximum temperature exceeded 55° F. or 13° C. for 3 days or longer. (The Illinois

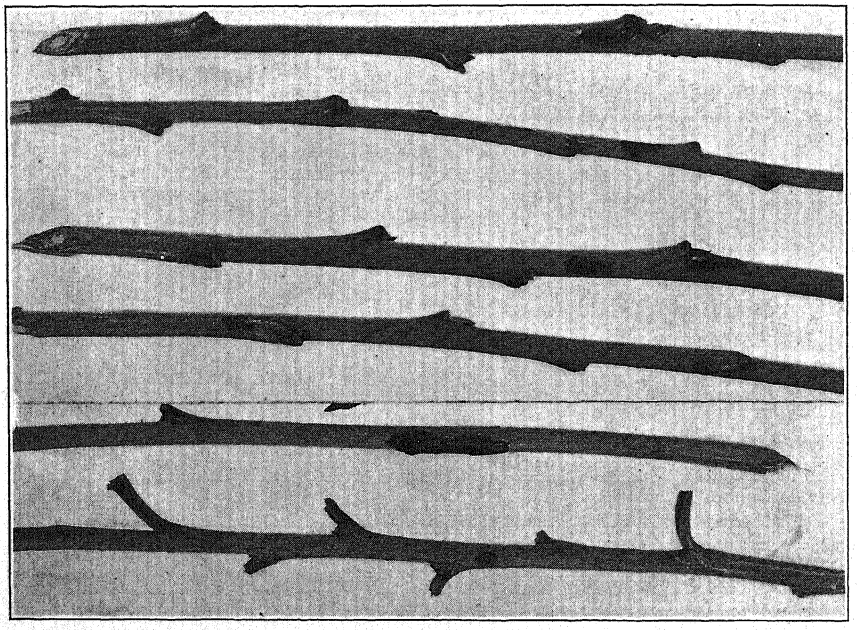


FIG. 5. Cankers produced from natural field infection on green shoots during the summer. Collected November 27, 1930.

section of The Climatological Data was the source of temperature records). In cultures kept in a refrigerator at $-2°$ to $+2°$ C. the organism was viable after 5 months. The optimum, minimum, and maximum temperatures for growth of the bacteria were found to be 25°, 7°, and 38° C., respectively. These were determined from 10 freshly inoculated dextrose-agar slants incubated at temperatures of 4°, 7°, 12°, 18°, 25°, 32°, 38°, 46°, and 52° C., for 10 days. No growth was evident outside the range of 7° to 38° C. The organism that had been held at 4° C. grew when incubated for 3 days at 25° C., while those at 46° and 52° C. were not viable. Thermograph records showed that the temperature varied less than 2° C. throughout the experiment. The thermal death point was found to be 52° C.

OVERWINTERING IN TWIGS

The pathogen survived the winter of 1931–32 at Yonkers, New York,[2] in peach twigs artificially inoculated at intervals of about 2 weeks, from November to June, inclusive. Needle-prick inoculations, consisting of 100 punctures into the cortical tissue of young twigs with culture No. 202, were made on November 9, 14, December 3, 15, 1931, January 1, 15, February 3, 15, March 1, 17, April 3, 15, May 2, 14, June 2, and 19, 1932. One hundred pricks with a sterile needle on each of these dates served as controls. On February 3, 2 of the inoculations of November 9 near the tip of a twig had developed typical blistery cankers about 5 mm. in diameter around the puncture. On April 15, 10 additional cankers appeared from the inoculation of November 9 and one from the February 15 inoculation. By May 22, one other canker appeared from the February 15 inoculation. At the last observation, August 18, no other cankers had developed. Thus, from a total of 1,600 inoculations, 14, or .875 per cent, developed typical spring cankers. However, 12 per cent of the inoculations of November 9 developed cankers. All the infections artificially produced were those near the tip of the twigs. In no case did infections result from the inoculations into 2-year-old twigs.

Primary leaf infections near the cankers were observed on May 22 when no infection was found elsewhere in the orchard.

Isolation plates from the leaf spots found May 22 and from the cankers on February 3, April 15, May 22, and August 18, all developed typical colonies of *Phytomonas pruni*. The last isolation, however, showed that the bacteria in the diseased tissue had decreased considerably in number during the summer. Stained microtome sections of the cankers collected and fixed on February 3 and April 15 contained bacteria in the diseased tissue.

ISOLATIONS FROM OVERWINTERING LEAVES

Since the observations made by Anderson (2) indicated that overwintering on fallen leaves was possibly the source of the primary inoculum, some additional attempts have been made to obtain positive evidence on this point. During the winter and spring of 1931–32 isolations of *Phytomonas pruni*, made from fallen leaves as late as February 8, indicate that the bacteria may survive in fragments of fallen leaves throughout the winter months. No direct evidence that the primary inoculum comes from this source, however, has been adduced.

[2] The results of this experiment are included in this paper with the permission of the Director of The Boyce Thompson Institute for Plant Research, Yonkers, New York.

DISCUSSION

The spring cankers differ from the summer twig cankers in the time of their development, appearance, and infection. The former develop on the tip portion of overwintering twigs and on water sprouts before green shoots are produced, while the latter develop only in the green tissue of the shoot. Inoculations of overwintering twigs throughout the spring until June 15, at the time when green shoots were susceptible, indicated that they were resistant. The twig inoculations during the fall and winter of 1931–32 showed that the pathogen, if introduced by puncture, can survive the winter in the twigs and produce typical cankers the following spring. The summer cankers develop from infections usually after foliage infection is well established. Both types of cankers contain abundance of bacteria when they are first formed, but during the summer they dry considerably and there is a marked diminution of bacteria. Throughout the following winter and spring it is difficult to isolate the pathogen from either type of canker. This difficulty has already been mentioned by Anderson (2) and Dunegan (3). The overwintering cankers of either type are not considered to be the principal source of inoculum for primary foliage infection. The cankers shown by Dunegan (3) in figure 6, B, appear quite similar to those in figure 5, which are known to develop early in the spring. Only the early stage of the spring cankers supply this inoculum.

Why *Phytomonas pruni* does not survive the summer in twig cankers may be explained from the conditions found in stained microtome sections. Very shortly after inoculation the host reacts by walling off the infected area by the formation of corky layers of cells, distinctly visible in all sections of cankered twigs (Fig. 2, B and D). If invasion occurs outside this layer, due to extreme activity of the bacteria, a second layer of cells forms (Fig. 6, A), and this process is repeated until the bacterial areas are successfully isolated. Any canker visible to the naked eye during the growing season is thus effectively separated from the active cortical tissue.

As the growth of the host tissue ceases in the late autumn any invasions resulting from late infections are not so isolated and may continue to develop additional bacterial cells and remain in intimate contact with the cortical cells. In the early spring before the host cells start division, the masses of bacteria invade the cortical region by the formation of zoogloeal masses, as described by Nixon and others in the case of *Erwinia amylovora*.

The older basal portion of the twig is more resistant to invasion than is the apical area. This probably is due in part to the fact that the stomata are older and less active and, in part, to the presence in the older portion of a phellem layer beneath the epidermis, which walls off the cortex against external invasion. The resistance of the overwintered twigs to invasion,

even when the cortex is punctured, may be explained by the compact and relatively inactive character of the cortical cells. Cankers have not been observed to develop on 2-year-old twigs. Also, infections failed to develop from inoculations into these twigs.

The incubation period for twig infections was found by Rolfs (6) to be from 4 to 10 days. Dunegan (3) found that it varied from 7 to 12 days and that cool weather prolonged the incubation period in leaf infections to 25 days. In twig inoculations throughout the past winter the period was prolonged to 86 and 157 days in the November 9 inoculations and from 59 to 96 days in the February 15 inoculations. The pathogen in culture remained viable when exposed to outdoor winter conditions and to ice-box conditions of $-2°$ to $+2°$ C. for 5 months. In a typical leaf lesion

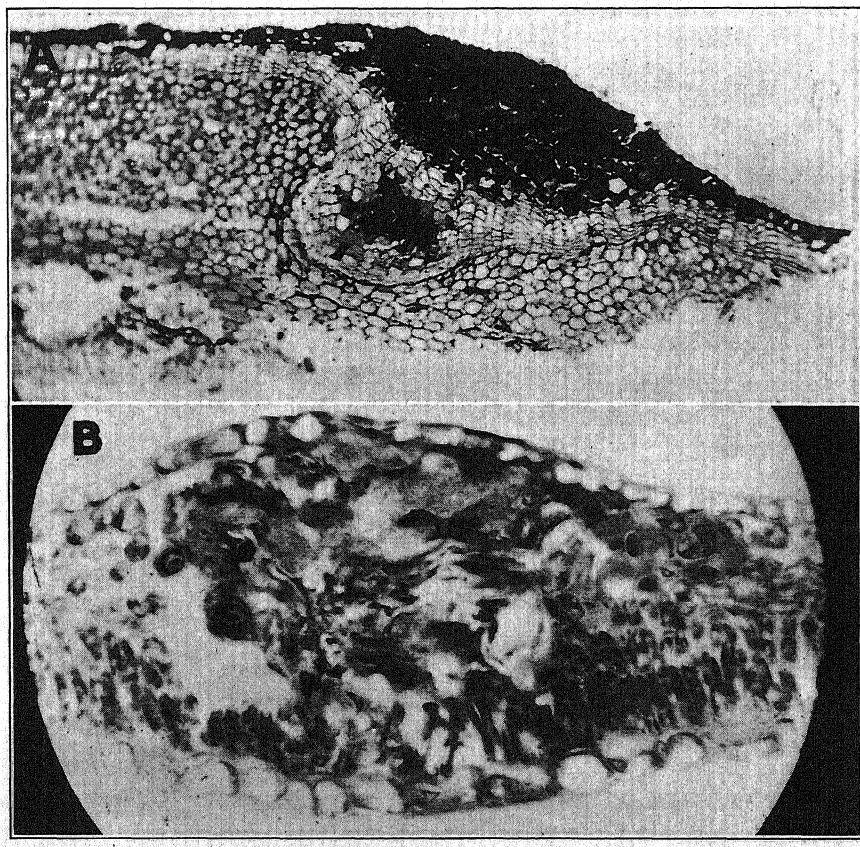


FIG. 6. Photomicrographs of stained sections. A. Secondary invasion, walled off by new layers of cambium. ×90. B. Bacteria in an Elberta leaf two months after inoculation in the greenhouse. ×740.

that developed in the greenhouse 2 months after inoculation, bacteria were visible in stained microtome sections. (Fig. 6, B.) During the incubation period was prolonged to 86 and 157 days in the November 9 inoculations rather low.

The temperature throughout the winter and spring appears to determine the time of development of spring cankers into visible lesions. Their appearance in southern Illinois seemed to be correlated with a mean maximum temperature of about 12° C. (53.6° F.) or higher for 5 or 6 days.

The time of twig infection is probably during rains before the infected leaves drop in the fall. After the organism has become established in the cortical tissue temperature seems to be the controlling factor for determining the incubation period. Noticeable lesions may not develop for 2 or more months after the initial infection, as was found in artificial inoculations into young twigs. It is believed that infections that become noticeable lesions or produce appreciable injury to the twigs before the growth is retarded by low temperature are corked off and are dried during the winter. Only late infections that occur shortly before the rest period of the host tissue starts constitute the source of canker bacteria the following spring.

SUMMARY

Spring cankers, a new type of twig lesions, were found in 1929. They develop on the previous summer's growth and on water sprouts shortly after the first leaf buds open.

Primary foliage infections have been correlated for 3 years with the spring cankers. Trees pruned of their 1-year and 2-year twigs, thus eliminating spring cankers, were not infected until bacterial spot was general in the orchard.

The pathogen was repeatedly isolated in 530 plates from young spring cankers. From overwintering cankers only one plate out of 370 developed *Phytomonas pruni* colonies. Isolations from young summer cankers were all positive, while those from overwintering cankers were negative.

The pathogen in culture survived the winter conditions of 1930–31 at Urbana and ice-box conditions of −2° to +2° C. for 5 months. Its optimum, minimum, and maximum temperatures for growth are 25°, 7°, and 38° C., respectively. Its thermal death point is 52° C.

The organism in artificial twig inoculations survived the winter conditions of 1931–32 at Yonkers, New York, and produced typical spring cankers the following spring.

DEPARTMENT OF HORTICULTURE,
AGR. EXPT. STA.
URBANA, ILLINOIS

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CONTROL OF GLADIOLUS SCAB

L. E. MILES

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Studies on the control of gladiolus scab caused by *Bacterium marginatum* were started in the spring of 1927 by Mr. H. H. Wedgworth, at that time Associate Plant Pathologist for the State Plant Board of Mississippi. The results secured by him in 1927 and 1928 were published in November, 1928, in Mississippi Bulletin No. 261, entitled Investigations on Diseases of Vegetable and Ornamental Plants. The writer took up the project in the fall of 1928 at the point where it was left by this publication. Four additional years of work have been devoted to it, so that it is now felt that fairly conclusive statements can be made relative to the control of this, the most prevalent disease of the gladiolus.

When this project was undertaken by Mr. Wedgworth, and, likewise when it was taken up by the writer, no work on a sufficiently comprehensive basis had been done with respect to it, so that, in consequence, there was great diversity of opinion as to the value of disinfection of gladiolus planting stock. When disinfection was practiced or attempted, the methods and materials used were even more varied and various. Treatments reported to the author as having been used with differing degrees of success have ranged all the way from coal soot through sulphur, the organic mercury dusts to liquid treatments such as Semesan, bichloride of mercury, and even to soap suds.

It was therefore considered desirable to secure some definite information as to the relative efficiency of at least some of the most promising from this multiplicity of materials and methods. With that end in view, the work begun by Mr. Wedgworth was continued and on a more comprehensive basis.

SOURCE OF MATERIALS

Letters explanatory of our plans and purposes were addressed to gladiolus growers in all sections of the country requesting their cooperation by donation of both diseased and healthy corms. A very hearty response was received to these requests and a large number of corms representing all degrees of infection was secured. The average condition of the diseased corms as used in the following tests with respect especially to the infection present is shown in figure 1. Many varieties were represented, but fortunately a large enough number of a single variety was usually available to make up the more important test plats. The author wishes here to express his thanks to all growers who rendered such invaluable assistance.

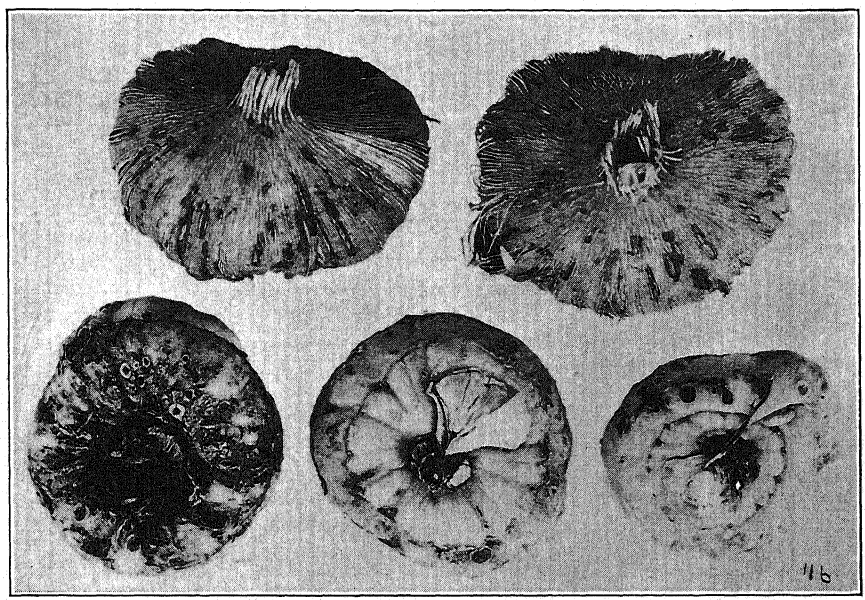


FIG. 1. Average conditions of corms planted in these tests as moderately or severely infected with scab.

Materials used in the treatment of corms and soil were in most cases contributed by the manufacturers. Thanks also are due to these companies for their generous cooperation.

EXPERIMENTS ON CONTROL OF GLADIOLUS SCAB BY CORM TREATMENT

In 1929 a test was designed to show the relative efficiency of various lengths of treatment with corrosive sublimate, Semesan, and formaldehyde. A single variety, America, was used in this test. The lengths of time of treatment for each of the disinfectants were 6, 8, 12, and 16 hours. The number of corms in each treatment was 125, and the test was repeated once, making a total of 250 corms per treatment. The corms were treated on March 18, 1929, and planted on the following day. Table 1 gives a summary of the results secured.

Examination of table 1 reveals that most efficient control of scab was secured from the 12-hour soak in mecuric chloride, followed next in order by the 8-hour soak, with the 16-hour treatment period only shortly behind it. The 6-hour period apparently was too short to give efficient results with this material. On the other hand, with Semesan, the 6-hour treatment gave the largest number of clean corms of any time period used. It was, however, below all treatments with bichloride of mercury except the 6-hour period.

TABLE 1.—*Effect of length of treatment with $HgCl_2$, Semesan, and formaldehyde, 1929*

Treatment	Length of time	Germination	Increase	Clean	Scabbed
	Hrs.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
$HgCl_2$ 1–1000	6	96.0	92.4	57.7	42.3
Semesan 1–400	6	92.8	101.4	84.1	15.9
Formaldehyde 1–240	6	92.0	27.6	32.0	68.0
$HgCl_2$ 1–1000	8	97.5	102.6	91.8	8.2
Semesan 1–400	8	90.4	106.0	77.7	22.3
Formaldehyde 1–240	8	100.0	23.0	64.9	35.1
$HgCl_2$ 1–1000	12	95.2	111.2	97.5	2.5
Semesan 1–400	12	88.8	127.6	83.3	16.7
Formaldehyde 1–240	12	94.9	33.2	28.1	71.9
$HgCl_2$ 1–1000	16	92.8	64.0	89.8	10.2
Semesan 1–400	16	96.0	100.7	76.0	24.0
Formaldehyde 1–240	16	92.3	25.4	41.7	58.3
Check	.....	96.4	48.7	6.5	93.5

Formaldehyde ranked far behind both these in the percentage of clean corms and was decidedly inefficient.

When the percentage increase in crop is considered, it is found that it follows in the main the percentage of clean corms harvested. Some variations do occur, as, for instance, the fact that the largest increase in crop is produced by the 12-hour treatment with Semesan, while that treatment ranks fifth in the percentage of clean corms produced. Formaldehyde produced even a smaller increase in crop than did the untreated lots, although the percentage of clean corms was very decidedly larger. Results shown in this table confirm the conclusion reached by Wedgworth that formaldehyde should not be recommended for the treatment of gladiolus corms against scab.

In 1930 an additional supply of corms was secured from growers and a comprehensive lot of tests designed and planted. A very severe and prolonged drought occurred, however, and affected the plats so severely that no results were secured. The dry period began just as the plants were beginning to bud and affected them to such an extent that many died in the field without blooming. Others bloomed but later died without making a new corm. Such corms as were harvested were almost entirely free of scab, regardless of the treatment received by the particular plat from which they came. It was evident, therefore, that the unusual weather conditions had held the disease in check to such an extent that all treatment results were vitiated and rendered worthless.

The 1930 series of tests were, therefore, in large measure, repeated in 1931 (Fig. 2). The 1930 crop of corms, being largely disease free, as a



FIG. 2. A partial view of the 1931 gladiolus-scab-control test plots, giving some idea of their extent.

result of the weather conditions referred to above was carefully sorted and used chiefly for checks, and new diseased materials were secured from the growers who had cooperated in previous years. In table 2 are presented the results of a test of the efficiency of bichloride of mercury, and the substances Calogreen and Semesan. Calogreen is a mercurous mercury compound, being in fact, a form of calomel. The corms were treated and planted on March 11 and 12, 1931. The variety, Ida Van, was used. Each treatment consisted of 100 corms and the treatments were replicated 4 times, making a total of 2,400 corms in all in the test.

This test shows Calogreen standing far in advance of any other material in its efficiency in scab control. Commercially, the corms harvested from

TABLE 2.—*Gladiolus corm treatments with $HgCl_2$, Calogreen and Semesan, 1931*

Treatment	Concentration	Time	Increase	Clean	Scabbed
			Per cent	*Per cent*	*Per cent*
Check (diseased)	.....	.....	28.5	6.4	93.6
$HgCl_2$	1–1000	8 hrs.	111.4	87.2	12.8
Calogreen	1 lb.–10 l.	5 min.	190.0	98.1	1.9
Semesan	1 oz.–12 l.	5 min.	108.9	78.8	21.2
Check (clean)	.....	.....	141.7	96.3	3.7

the plat treated with this material would have been considered 100 per cent clean and the increase in the weight of crop harvested was far beyond any other treatment, although the clean check gave an abundant harvest. Bichloride of mercury and Semesan ranked very much the same as they had done in previous tests but both were much less efficient than was Calogreen.

Another test was planned to try out the efficiency of Calogreen at different concentrations. The rate previously tested, 1 pound to 10 liters of water, was used again, and with it was compared a concentration only one-half as great, namely, ½ pound to 10 liters of water. In this test was used also an organic mercury product designated as 80–A, provided for the purpose by the Bayer-Semesan Company. It was used at the rate of 1 pound to 12 liters of water. Calogreen and 80–A formed thick suspensions and the corms were allowed to remain in them for a period of 5 minutes. Frequent agitation was practiced to keep the materials thoroughly in suspension and to assure that all the corms were completely in contact with the chemicals. All of these short soaks were again checked against a soak of 8 hours in a 1–1000 solution of bichloride of mercury, which was used throughout all of these tests as a standard for comparison. Diseased corms only were used as a check. One hundred corms of the variety Le Marechal Foch were used for each treatment. The treatments were quadruplicated, thereby making a total of 2,000 corms in the entire test. A summary of the results secured is presented in table 3.

TABLE 3.—*Summary of short-soak treatments compared with 8-hour soak in 1–1000 $HgCl_2$, 1931*

Treatment	Concentration	Time	Clean	Severe scab	No. 1
			Per cent	*Per cent*	*Per cent*
Check (diseased)	.....	.....	0.0	91.1	13.2
$HgCl_2$	1–1000	8 hrs.	80.8	3.4	40.8
Calogreen	1 lb.–10 l.	5 min.	96.8	0.0	71.0
Calogreen	0.5 lb.–10 l.	5 min.	90.5	0.9	63.2
80–A	1 lb.–12 l.	5 min.	61.2	22.7	23.8

Both concentrations of Calogreen gave efficient control. The stronger concentration, however, gave a higher percentage of clean corms and also a greater number averaging No. 1 in size. The product, 80–A, did not give efficient control of scab at the concentration used. This test agrees with the one previously reported in placing Calogreen first in rank in efficiency of all materials tested.

The next test to be reported consisted of a comparison of 2 treatments with a substance sold under the trade name Calochlor, using again an 8-hour soak in a 1–1000 solution of bichloride of mercury as a standard for comparison. Calochlor is reported to be a mixture of mecurous and mecuric chlorides. It was used at a concentration of 3 ounces to 5 gallons of water. One lot of corms was soaked for 5 minutes and another for 30 minutes. Almost constant agitation was necessary to keep the Calochlor in suspension. The variety Alice Tiplady was used in this test, at the previous rate of 100 corms per treatment. The treatments were again quadruplicated, making a total of 1600 corms in this test. The results secured are summarized in table 4.

TABLE 4.—*Comparison of 8-hour soak with $HgCl_2$, 1–1000, with Calochlor, 5-minute dip and 30-minute soak, 1931*

Treatment	Concentration	Time	Clean	Severe scab	No. 1
			Per cent	*Per cent*	*Per cent*
Check	.....	.....	2.1	93.8	14.1
$HgCl_2$	1–1000	8 hrs.	91.1	1.2	83.0
Calochlor	3 oz.–5 gals.	5 min.	62.0	19.2	44.1
Calochlor	3 oz.–5 gals.	30 min.	69.9	17.7	33.8

Neither of the Calochlor treatments shows in this test a sufficiently high degree of efficiency to warrant its further use. Germination was delayed and the flower spikes were shorter than in any other plot in the entire series of tests. This was especially marked in the 30-minute-soak treatment.

Reports from various sections of the country have indicated that a short soak with a solution of Gold Dust has sometimes given effective control of scab. Others have reported that a 2-hour soak in a solution of acetic acid has been satisfactory. It was, therefore, thought advisable to test these 2 methods against the 8-hour soak in 1–1000 bichloride solution. The Gold Dust was used at the rate of 6 ounces to 10 liters of water and the time period set at 45 minutes, which was a compromise between the 30-minute and 1-hour periods seemingly in most frequent use. Acetic acid of 56 per cent concentration was used at the rate of 8 pounds to 50 gallons of water for a period of 2 hours. 100 corms of a single variety, Evelyn Kirtland, were used in each treatment, and the test was triplicated, making a total of 1200 corms in all. The summary of results secured is presented in table 5.

Examination of the table will show that both acetic acid and Gold Dust proved decidedly inefficient in this test, the harvested crop of corms being

TABLE 5.—*8-hour soak in 1–1000 $HgCl_2$, 2-hour soak in acetic acid, and 45-minute soak in Gold Dust, 1931*

Treatment	Concentration	Time	Clean	Severe scab	No. 1
			Per cent	*Per cent*	*Per cent*
Check (diseased)	.....	.....	0.0	98.1	7.6
$HgCl_2$	1–1000	8 hrs.	79.2	7.2	68.0
Acetic acid	8 lbs. 56%–50 gals.	2 hrs.	3.9	89.7	17.9
Gold Dust	6 oz.–12 l.	45 min.	1.4	92.3	22.8

in but little better condition than that from the nontreated diseased check plat. The Evelyn Kirkland corms used in this test were small, mostly numbers 2 and 3, and were very severely scabbed. Even at that, however, the 8-hour soak with 1–1000 bichloride gave a fairly clean crop.

Having available a considerable quantity of corms in relatively small number, that is, varying from 100 to 500 corms per variety, a test was designed to determine whether there was a possible difference in response to a particular treatment between different varieties. Seven varieties were divided into lots of 100 corms each, the number of such lots varying with the number of corms of the variety available. Each lot of 100 corms was again divided into 2 lots of 50 corms each. One of these was not treated, and the other was treated for 5 minutes in a solution of Calochlor, made up at the rate of 3 ounces to 20 liters of water.

TABLE 6.—*Varietal response to 5-minute soak in Calochlor, 3 ounces to 20 liters, 1931*

Variety	Treatment	Number of lots	Clean	Severe scab
			Per cent	*Per cent*
Le Marechal Foch	Check	1	3.6	88.9
" " "	Treated	1	70.0	13.9
Los Angeles	Check	8	1.1	93.9
" "	Treated	8	59.8	19.9
Merton W. Wentworth	Check	1	0.0	100.0
" " "	Treated	1	48.2	26.1
Mildred Adeline	Check	1	8.1	79.0
" "	Treated	1	74.4	6.8
Mrs. Dr. Norton	Check	1	2.9	87.8
" " "	Treated	1	60.7	20.1
Myra	Check	6	3.1	79.9
"	Treated	6	66.2	17.6
Myrtle	Check	1	1.2	91.0
"	Treated	1	68.4	16.7

In this case the choice of the disinfectant proved an unfortunate one. Calochlor, in harmony with the data recorded in table 4, gave unsatisfactory results as a disinfectant for the control of gladiolus scab. It would seem, however, from this test that there is no marked difference in the response of different varieties to the action of the disinfectant. As in a previous case where Calochlor was used, all varieties showed delayed germination and a stunted condition of the plant, which was never overcome. When the corms of the Merton W. Wentworth variety were examined after harvest, it was found that a considerable number of them were affected with Fusarium rot, in addition to scab. The presence of this additional disease may be in part responsible for the low percentage of clean corms secured in the case of this variety.

A second test, similar to the one just recorded, included only 6 varieties. The treatment consisted of a 5-minute soak in a suspension of Calogreen made up at the rate of 1 pound to 10 liters of water. Results are recorded in table 7.

TABLE 7.—*Varietal response to 5-minute soak in Calogreen, 1 pound to 10 liters, 1931*

Variety	Treatment	Number of lots	Clean	Severe scab
			Per cent	*Per cent*
Myrtle	Check	1	2.0	92.1
"	Treated	1	97.3	0.0
Rose 1910	Check	2	0.0	87.3
" "	Treated	2	93.9	0.0
Odin	Check	3	6.5	81.6
"	Treated	3	99.2	0.0
Pink Beauty	Check	6	0.0	100.0
" "	Treated	6	92.0	0.0
Schwaben	Check	5	1.3	90.6
"	Treated	5	94.9	0.0
Souvenir	Check	2	10.1	69.3
"	Treated	2	98.3	0.0

In this case, again, Calogreen proved to be very efficient. The most severely infected corm in any of the lots treated with it would ordinarily be passed by the commercial grower as perfectly clean. No corm treated with Calogreen at the rate of 1 pound to 10 liters of water has been found in any of these tests that has been either moderately or severely infected with scab.

Taking into consideration the results secured in the last 2 tests recorded, it may be reliably concluded that there is no difference in the response of a particular variety to the action of the disinfectant used. A total of 13 varieties has been tested with no evidence of such response.

Having some land available on which diseased gladioli had been grown in previous years, some tests were designed to get some information regarding a possible effect of disinfectants added to the soil about the corms on the prevention of infection by the soil-borne organisms. The plots chosen were two in which diseased crops of corms were grown in 1928 and 1929, respectively. No gladioli were ever grown in either of them except the crop hereinbefore mentioned. Four substances were used for soil treatment, namely, Calochlor, Calogreen, Semesan, and lime. The latter was used because of the claim made by a grower that he had repeatedly grown a clean crop of corms on infested soil by adding lime to the soil in the row. Four 24 ft. rows constituted a single plot. One row between each 2 plots and one on the outside of the end plots, 5 rows in all, left untreated, constituted the check plot. The Calogreen, Calochlor, and Semesan were applied at the rate of 6 ounces per 1000 feet of row, or 21.8 grams per 24-foot row. The lime was applied at the rate of 2 tons per acre, or 2.2 pounds per 24 foot row. The disinfectants and lime were mixed with the soil immediately adjacent to and above the corms. A total of 25 varieties was used in this test, 50 corms of a single variety constituting one row. All corms were severely infested with scab and all were soaked for 8 hours in a 1–1000 solution of bichloride of mercury. Results of the tests are summarized in table 8.

TABLE 8.—*Treatments of infested soil. Scabby corms treated 8-hours in 1–1000 $HgCl_2$, 1931*

Treatment	Diseased crop in 1928		Diseased crop in 1929	
	Clean	Severe scab	Clean	Severe scab
	Per cent	*Per cent*	*Per cent*	*Per cent*
Calochlor	88.3	4.0	79.9	7.1
Calogreen	95.1	0.0	91.3	0.3
Lime	31.0	24.2	40.1	37.6
Semesan	63.2	11.6	58.9	15.2
No Soil Treatment	48.8	26.3	45.0	30.9

Examination of this table reveals that soil infestation from both the 1928 and 1929 crops is abundantly in evidence. The percentage of clean corms on the soil that received no treatment is much lower than that to be expected from an 8-hour soak in 1–1000 bichloride of mercury, when the corms are grown in clean soil. The percentage of severely scabbed corms is much higher than when similarly treated corms are grown in soil where gladioli had not been grown previously. There is also some evidence to show that soil infestation is somewhat greater on soil where the diseased crop was grown most recently.

Lime had no effect in controlling infection from soil-borne organisms. In fact, in both cases the percentage of clean corms was smaller on the limed plot than on the plots that received no soil treatment. The excess amount of lime present also retarded the growth of the plants and greatly reduced the yield. Calochlor and Semesan were apparently somewhat effective in reducing infection from the soil, although Calochlor was much the less efficient of the two. Calogreen would seem to have been extremely efficient in preventing such infection. The percentage of clean corms was as high as, or even higher than, would be expected to have resulted from the 8-hour soak in bichloride of mercury, even when the crop is grown on disease-free soil.

Another test was designed to determine the efficiency of the same materials in preventing infection from soil-organisms when clean corms were used. The soil chosen was that on which a diseased crop had been grown in 1929. In this test 100 corms constituted a row. The soil in 2 rows was treated with one of the materials and that of the next 2 rows was not treated and served as a check. The soil of the next 2 rows was treated with another of the materials, with the corresponding pair of rows as a check, and so on throughout the test plot. The materials were applied to the soil at the same rate as in the preceding test. The variety, America, was used for the Calogreen-treated plot and its corresponding check, and Chicago White, E. J. Shaylor, and Sunbeam for the lime, Calochlor and Semesan plots, respectively. The data secured are recorded in table 9.

TABLE 9.—*Treatments of infested soil, 1931. Corms clean, nontreated*

Treatment	Clean	Severe scab
	Per cent	*Per cent*
Calogreen	72.3	8.6
Lime	8.8	63.0
Calochlor	58.0	20.7
Semesan	22.1	40.7
Check	16.2	48.1

Examination of table 9 reveals the fact that a heavy soil infestation was present in the plots, as shown by the high percentage of severely scabbed corms harvested, even though apparently disease-free corms were planted. Again, the limed plot yielded more severely infected and fewer clean corms than did the nontreated, check plot. Both Semesan and Calochlor proved relatively inefficient in preventing infection from soil-borne organisms, when applied to the soil; and again, Calogreen showed its versatility as a disinfectant for use against gladiolus scab. It yielded by far the highest

percentage of clean corms of any treatment tested and likewise the smallest number of severely scabbed corms. It would seem, therefore, that Calogreen, applied to the soil immediately adjacent to the corm, might have an important place in scab control for the small grower whose available land is too limited to allow of proper rotation to avoid the use of infested soil.

EXPERIMENTS ON SPRAYING AND DUSTING FOR SCAB CONTROL

Since many growers have reported excellent results from the use of Bordeaux-mixture sprays and copper-lime dusts in the control of scab and other diseases of the gladiolus, it was considered advisable to test these methods of procedure. The writer used a 4–6–50 Bordeaux mixture, consisting of 4 pounds of copper sulphate, 6 pounds of hydrated lime, and 50 gallons of water. Since the gladiolus leaf is smooth and of such texture that spray does not readily stick to it, a spreader, composed of resin fish oil soap at a rate of 3 pounds to 50 gallons of spray, was added. The spreader gave the spray liquid excellent sticking and spreading qualities and was quite efficient. The dust applied was a home-made product consisting of 20 per cent monohydrated copper sulphate and 80 per cent of hydrated lime.

Three plots of 400 corms each of the variety Pink Beauty were used for this test. One plot was sprayed at intervals of 2 weeks with Bordeaux-mixture, beginning when the plants were 4 to 6 inches above the ground. Another plot was dusted with copper-lime dust on the same dates, and the third, a nontreated plot, served as a check. Six applications of both spray and dust were given, the last being shortly after the blooming period but before the plants had begun to turn yellow. The dust was applied in the early morning, while the plants were wet with dew. The variety Pink Beauty was chosen for this test because in previous years it had shown probably more foilage infection than had any other variety observed. The corms were all severely infected and averaged No. 2 in size. They were planted in soil in which gladioli had not been grown previously. A total of 200 corms in each plot was soaked for 8 hours in 1–1000 bichloride of mercury, and the remaining 200 were not treated. The results secured are presented in table 10.

Examination of table 10 shows that Bordeaux-mixture almost entirely eliminated foliage infection on plants from treated corms. When severely diseased corms were not treated prior to planting foliage infection was greatly reduced but not entirely eliminated. Even on the plants that did show foliage infection, the infection was much less severe than on the nonsprayed plants. The copper-lime dust was much less efficient than the Bordeaux-mixture spray. It is unfortunate that the test was not repeated on land known to be infested with the scab organism. This, however, was not done, since such land was not available at the time of the test. It is

TABLE 10.—*Spraying and dusting gladioli for scab control, 1931*

Treatment	Corms treated 8 hours in 1–1000 $HgCl_2$		Corms nontreated	
	Plants with foliage infection	Clean corms	Plants with foliage infection	Clean corms
	Per cent	*Per cent*	*Per cent*	*Per cent*
Check (nontreated)	28.9	73.0	88.7	0.0
4–6–50 Bordeaux mixture	0.9	84.2	16.6	34.6
20–80 Copper-lime dust	11.3	70.3	52.1	16.2

evident from the results secured that spraying, alone, will not give a satisfactory control of scab when severely diseased corms are planted, even on noninfested soil. More tests must be made before a satisfactory conclusion can be reached as to whether it will be commercially practicable to spray plants from corms that have been given an efficient treatment prior to planting.

SUMMARY

Calogreen proved by far the most efficient material tested as a disinfectant agent against the scab organism on gladiolus corms.

A 1–1000 concentration of bichloride of mercury ranked next in efficiency. A 12-hour treament with this solution gave best results. The 8-hour treatment, however, gave almost as good control.

Semesan, an organic mercury product, was fairly efficient in the control of scab, ranking somewhat below bichloride of mercury.

Formaldehyde, acetic acid, and Gold Dust were decidedly inefficient as corm disinfectants.

Calochlor, although it did reduce the amount of scab to a fair degree, delayed germination, retarded growth of the plant, and resulted in a considerable reduction in the crop of corms harvested.

There is, apparently, no difference in the response of any particular variety of gladiolus to the efficiency of the disinfectant used.

Calogreen, applied to the soil on land known to be infested, proved efficient in reducing infection from soil-borne scab organisms. Calochlor reduced such infection but delayed germination and retarded growth. Semesan gave but fair protection and lime was decidedly inefficient.

Bordeaux mixture proved notably more efficient in the prevention of foliage infection than did copper-lime dust.

DEPARTMENT OF PLANT PATHOLOGY,
MISSISSIPPI AGRICULTURAL EXPERIMENT STATION
STATE COLLEGE, MISSISSIPPI.

POWDERY MILDEW OF CRAPE MYRTLE CAUSED BY ERYSIPHE LAGERSTROEMIAE, N. SP.

ERDMAN WEST

(Accepted for publication, Dec. 1, 1932)

Powdery mildew of crape myrtle was first reported[1] in the United States in 1924 by J. J. Taubenhaus, who found it in 2 counties in Texas. Since then it has been reported[2] in six other Southern States (South Carolina, Georgia, Alabama, Louisiana, Mississippi and Florida) and the District of Columbia. The first collection in Florida was made at Gainesville in 1925 by G. F. Weber, but it has been found subsequently at widely separated points and its range in this State is probably coextensive with that of its host. Boyd reported it once on *Lagerstroemia parviflora* in Georgia but all other collections have been made on *L. indica.*

Although the disease is of variable importance from year to year, it is usually prevalent enough each season to cause a conspicuous disfiguration of crape myrtle plants. (Fig. 1, A.) This is particularly true of plants in shaded situations and in hedges. From Alabama and Georgia we have reports of instances where one hundred per cent of the leaves were affected. This is unusual, but cases where one third or more of the leaves were mildewed have been observed frequently in Florida.

The first indication of the presence of the fungus occurs in early spring before the new shoots are more than a few inches long. At this time a few abortive shoots are found projecting barely beyond the bud scales and covered with a white coating of conidia. (Fig. 1, C.) These minute deformed shoots are very inconspicuous and easily overlooked. They serve as the foci for the general infection of the plants. They also indicate, in all probability, how the fungus is able to overwinter without the interpolation of a perfect stage in its life history. Protected by the bud scales during the period that the host is dormant and leafless, the fungus is able to resume growth with the advent of spring and the appearance of succulent susceptible growth on the host.

The first conspicuous growth of the fungus occurs on the young leaves as small, circular, white patches in which the mycelial strands radiate from the center. By the time that the spot is a centimeter in diameter, conidia are being produced in the center of the spot. After a few leaves or one

[1] United States Department of Agriculture. Bur. Plant Indus. Plant Dis. Rptr. Sup. 42: 349, 1925. (Mimeographed).

[2] United States Department of Agriculture. Bur. Plant Indus. Plant Dis. Rptr. 9: 12, 50, 1925. 10: 22, 1926. 11: 56, 106, 1927. 13: 110, 1929. 15: 103, 1931. Plant Dis. Rptr. Sup. 50: 448, 1926. 65: 425, 1928. 73: 387, 1929. 75: 71, 1930. (Mimeographed).

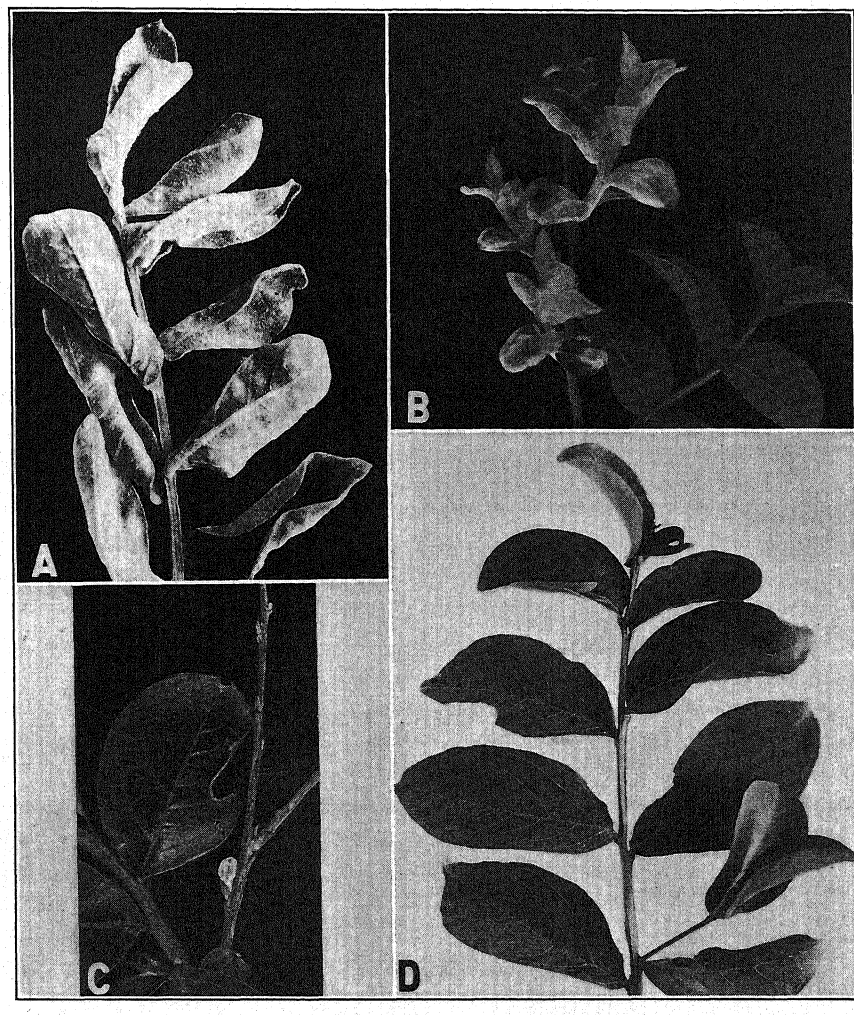


FIG. 1. A–C. Mildew on crape myrtle. A. Attacked shoot. B. Young shoot covered with spores. C. Heavily infected, aborted shoot. D. Healthy shoot.

shoot has become covered with the mildew (Fig. 1, B), the whole plant may show general infection in a week or ten days under favorable conditions. The conidia are produced in great numbers and the spread of the disease is rapid.

The disease appears first on the young shoots in early spring and continues to affect the plants visibly until the flowering spikes appear. With the advent of hot weather of midsummer, the disease apparently disappears from the plant. However, typical specimens can be found, at this time, on young sprouts near the center of the plant and on the foliage near the

ground. As fall approaches, the disease again appears on the young shoots on the outer portion of the plants and also frequently covers the older leaves as well. It is present on the plants until the leaves drop in December in Florida.

The plant parts attacked include leaves, young stems, and the various parts of the inflorescence. The affected organs soon become covered with a heavy, white, mealy coating, which is quite dusty from the innumerable spores present. When infection occurs on young organs, considerable malformation takes place. Young leaves are stunted before they attain a third of their normal size; at the same time they may become abnormally thickened. Young stems of shoots are stunted, the internodes are short and the stems irregular. When the young inflorescence is attacked, pedicels and peduncles are shortened and the buds usually fail to open. Quite frequently the diseased portions of the plant assume an unnatural reddish color beneath the white, mealy fungous growth. The diseased leaves and buds usually drop in a week or two but diseased stems frequently sprout and continue normal growth, especially when hot weather ensues.

When older leaves and stems become infected, there is little or no malformation of the organs. The leaves may even remain attached to the stem until the mealy coating weathers off, but they usually drop soon after the entire surface has been attacked by the fungus.

The organism causing crape myrtle mildew belongs to the Erysiphaceae. All reports so far published indicate that only the imperfect or oidium stage has been found in the United States. In the case of this fungus, as with other powdery mildews, the conidial stage is the conspicuous and injurious form of the organism. The description given above is that of the disease caused by this imperfect stage. However, the perfect stage of the fungus has been found once in Florida.

While the disease had been observed a number of times in Florida in 1925, no perithecia of the fungus had been observed nor had any particular effort been made to find them. Beginning with the first appearance of the mildew in the spring of 1926, the writer made frequent and careful examinations of diseased leaves, stems, and floral parts on a row of crape-myrtle bushes growing in the experimental plots of the University of Florida. As the season progressed, nothing but the conidial stage was observed. Not only were the living infected parts observed, but examinations were made of fallen and dead leaves, ripened stems and the inflorescences after flowering was past. The scanty infection during the hot weather was carefully examined, as well as the heavier infection which followed later.

Observations were continued on the brownish, mycelial coat which persisted on the older leaves and stems and early in December the ascogenous form was found. A single leaf bearing three mature perithecia was found

first and a few more before the leaves fell. In spite of exhaustive searching, no more perithecia have been found since that time, either on these particular plants or on affected plants in other localities.

In the conidial stage species of powdery mildews are not distinguishable and until perithecia are found, their exact taxonomic position must remain in doubt. The only powdery mildew bearing perithecia on crape myrtle has been *Uncinula australiana* McAlpine, reported only from Australia and Japan.[3] Therefore, previous American collections of powdery mildew on crape myrtle have been referred to this species. The perithecia of the Florida organism bear no trace of curling at the tips of the appendages, such as is characteristic of the genus Uncinula. The appendages in our species are short, erect, unmodified at the tip and distinct from the hyphae. In general, it resembles some forms of *Erysiphe polygoni* DC. in Florida, but has much smaller spores that measure only 14–17 μ long and 5–7 μ broad. Since no previously reported species has been found corresponding to these measurements and characteristics, the organism collected in Florida is therefore described as a new species.

Erysiphe lagerstroemiae, nov. sp.

Amphigenous; mycelium of two types, one evanescent and the other persistent; the evanescent type hyaline, shining and disappearing with the decline of the host, 7–9 μ in diameter; the persistent type remaining until late fall, septate, light to deep brown, rather thick-walled, frequently branched, 4–5 μ in diameter, associated with the perithecia.

Perithecia (Fig. 2, A) widely dispersed, never gregarious, globose, frequently, although not always, with a slight papilla-like knob on top, about 90 μ in diameter but varying from 80–100 μ; cells small, dark, distinct, 6–8 μ in diameter; appendages short, equalling the diameter of the perithecia in length, 90–110 μ long, 7 μ in diameter, colored yellow-brown throughout, not sharp-pointed, sometimes very slightly enlarged at point of attachment, not crowded, 6–15 in number, mostly straight or a few slightly flexuous, not interwoven with the mycelium; the perithecia attached at the base by 15–20 strands of brown, septate, flexuous hyphae of the persistent type; asci many (Fig. 2, B and C), varying from 10–23, average 16, small, ovate or broadly ovate, sometimes with a very short stalk, 30–44 μ × 17–20 μ; spores 6–8 usually 8, ovate or longer, broadest in middle, 14–17 μ × 5–7 μ; conidia borne in great numbers on evanescent mycelium, barrel-shaped, variable, hyaline, 30–34 μ × 14–17 μ.

[3] Salmon, E. S. A Monograph of the Erysiphaceae. Torr. Bot. Club Mem. **9**: 1–292. p. 118. 1900.

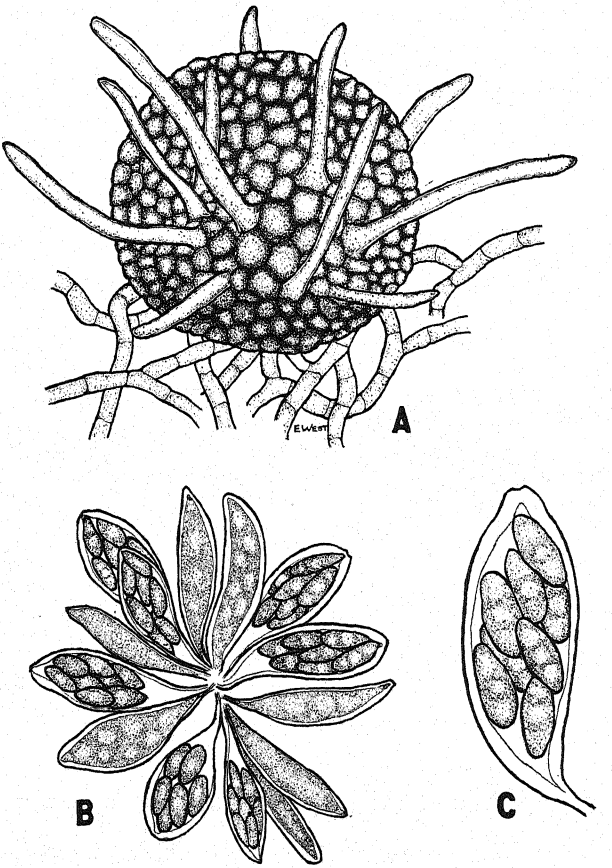


FIG. 2. *Erysiphe lagerstroemiae.* A. Perithecium showing appendages. B. Group of asci squeezed from perithecium. C. Mature ascus containing eight ascospores.

Host *Lagerstroemia indica* L.

Distribution: Texas, Louisiana, South Carolina, Georgia, Alabama, Mississippi, Florida, District of Columbia. Perfect stage reported from Florida only.

CONTROL

It has been found that a single application of commercial lime-sulphur spray diluted 1:80 will give a satisfactory control of crape-myrtle mildew. This spray has been applied in the past whenever the powdery mildew became apparent on the foliage. Since the discovery of the overwintering stage in the buds, it is recommended that the spray be applied as soon as the buds burst in the spring, so that a general infection of the plants may be prevented.

SUMMARY

Crape-myrtle mildew, in Florida at least, is caused by a new species of fungus herein described as *Erysiphe lagerstroemiae,* nov. sp.

It is probable that this organism is responsible for the same disease in several other Southern States.

The disease is characterized by the type of injury generally associated with powdery mildews.

The perfect stage of the organism is produced only infrequently.

The fungus is capable of overwintering in the leaf buds of the host.

The disease can be controlled with commercial lime-sulphur spray.

DEPARTMENT OF PLANT PATHOLOGY,
FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

RECORDS OF CITRUS CANKER FROM HERBARIUM SPECIMENS OF THE GENUS CITRUS IN ENGLAND AND THE UNITED STATES

H. S. Fawcett and Anna E. Jenkins[1]

(Accepted for publication Nov. 14, 1932)

INTRODUCTION

For the purpose of obtaining more information on the occurrence and distribution of the disease known as citrus canker, caused by *Pseudomonas citri* Hasse (2), material of the genus Citrus (mostly cultivated) was examined in 1930 at the Herbaria of the Royal Botanic Gardens, Kew, and the Department of Botany, British Museum, South Kensington, London, England, and, in 1931, at the Gray Herbarium, and Arnold Arboretum, Harvard University, Cambridge, Massachusetts; New York Botanical Garden, New York, New York; the Philadelphia Academy of Natural Sciences, Philadelphia, Pennsylvania, and the United States National Herbarium, Washington, D. C.

Tabulations and discussions of data obtained from this study are here presented, for it is thought that they may be helpful in tracing some of the history and distribution of the disease. The history and geographical distribution of citrus canker are discussed by Tanaka (7), Peltier and Frederich (6), Fawcett and Lee (1), and others.

DATA OBTAINED FROM PHANEROGAMIC SPECIMENS

In table 1 are listed the phanerogamic specimens on which canker, or what is apparently canker, was observed. These data appear to add no new countries to the distribution of the disease.

Of the Philippine specimens 1 was collected in 1914, or the year the seriousness of the disease in the Philippines became apparent (8), although it had not yet been distinguished as canker and not scab, 4 specimens were collected the following year when the disease was actually recognized as canker (9), the others at somewhat later dates. Figure 1, A, represents one of these specimens.

The specimens of *Citrus medica* from northwestern India, possibly 1827–1831 (Fig. 1, B), and *C. aurantifolia* from Java, 1842–1844 (Fig. 1, C), however, furnish by far the earliest records yet known for the disease.

[1] Part of a paper read at the annual meeting of The American Phytopathological Society, December 30, 1931 (3). The remainder of the paper, dealing with citrus scab, has been presented separately (4). Thanks are due A. W. Hill, A. D. Cotton, W. R. Maxon, C. E. Kobuski, F. W. Pennell, J. Ramsbottom, E. M. Wakefield, and A. C. Weatherby for courtesies in connection with the examination of the phanerogamic specimens.

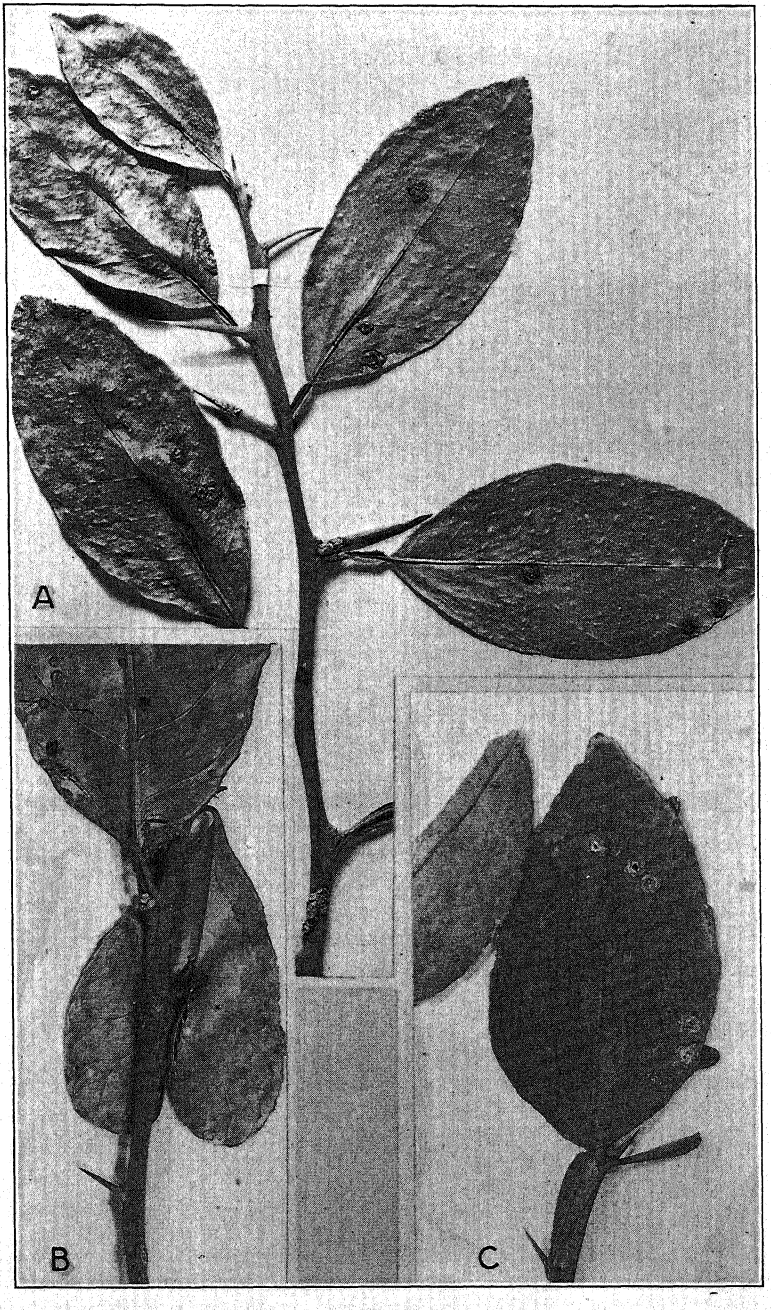


FIG. 1. Citrus canker on dried phanerogamic specimens of (A) *Citrus limetta* var. *aromatica*, Philippines, (Wester 2182); (B) *C. medica*, N. W. India; (C) *C. aurantifolia*, Java. ×1. A, photographed by M. L. F. Foubert; B and C, at Kew Herbarium.

TABLE 1.—*Identification of citrus canker or what is apparently citrus canker on phanerogamic specimens of the genus in herbaria in England in 1930 and the United States in 1931*

Herbarium	Data on specimens				Name of collection, number of specimen and collector, where given	Identification (asterisk (*) indicates that fragments of specimen showing lesions, are preserved in the Mycological Collections of the Bureau of Plant Industry)
	Date of collection	Place of collection	Kind of citrus			
			Tanaka's identification, where given	Name on label		
The Herbarium, Kew England	Early, possibly 1827–31[a]	N. W. India	*C. medica* L.	*C. medica* L.	Herbarium Royle "Herbarium Hookerianum 1867"[1]	Canker on leaves and twigs (Fig. 1, B)
"	No date on specimen, but reference to it by Moritzi (5) shows that it was collected between 1842–44	Java	*C. aurantifolia* Sw.	*C. javanica* Bl.	Plantes javanicae a cl. Zollingers lectae No. 555. In Herbarium Benthamianum, 1854	Canker on leaves. Notation on sheet shows that Tanaka also observed the numerous lesions of citrus canker on this specimen. (Fig. 1, C)
U. S. National Herbarium, U. S. A.	1903	Java	*C. hystrix* DC.	*C. hystrix* DC.	Herb. Bu. Agr. Manila No. 111, G, 5	?Canker on leaves*
"	Feb. 3, 1914	Philippines	*C. aurantifolia* (Christm.) Sw.	*C. lima* Zunan	Merrill: species blancoanae No. 436	Canker leaves severely affected*
"	March, 1915	"	"	*C. limetta* var. *aromatica* Wester	Flora of the Philippines 741–23 P. J. Wester	?Canker on leaves*

TABLE 1—(*Continued*)

U. S. National Herbarium, U. S. A.	March, 1915	Philippines	*C. aurantifolia* (Christm.) Sw.	*C. limetta* var. *aromatica* Wester	Flora of the Philippines 741–23 P. J. Wester	twigs (Fig. 1, A) Canker on leaves and
Arnold Arboretum, U. S. A.	"	"	"	*C. pseudolimonum* Wester	" 1953 P. J. Wester	Canker on leaves*
U. S. National Herbarium, U. S. A.	"	"	*C. excelsa* Wester	*C. excelsa* var. *davanenses* Wester	" 1009 P. J. Wester	Canker on leaves*
"	July, 1916	"	*C. medica* L.	*C. "limonum"* of Wester	" 116 P. J. Wester	Canker on leaves*
"	Sept., 1916	"	*C. aurantifolia* (Christm.) Sw.	*C. aurantifolia* Sw.	" (Forestry Bureau No. 26, 278)	?Canker on leaves*
"	1917	Asia	*C. depressa* Hayata	*C.* "the tachibana" *nobilis* var. *spontanea* Ito and Matsum	Arnold Arboretum Expedition to Eastern Asia 1917–18. Luikiu Islands No. 8049	Canker on leaves*
Arnold Arboretum U. S. A.	Apr. 27, 1919	Philippines	*C. hystrix* DC.	*C. micrantha* Wester	Flora of the Philippines (Forestry Bureau No. 27, 436)	Canker on leaves.* Lesions on same specimen as presented in U. S. National Herbarium are apparently those of canker.*

[a] Date of possible collection by Royle as suggested in letter from Dr. R. N. Parker, Forest Research Institute, Dehra Dun, U. P. India For further discussion see text.

Notations on the Javanese specimen indicate that the canker lesions on this specimen were observed by Tanaka, presumably in 1928, when he identified it as *C. aurantifolia.* In correspondence regarding the specimen from the Royle Herbarium, collected in northwest India, contained in the Herbarium Hookeriarum, Dr. R. N. Parker, Forest Botanist at the Forest Research Institute, Dehra Dun, has recently suggested that this may be Royle 7. He adds, "It is probable that Royle collected his specimen of *Citrus medica* in one of the ravines near Dehra Dun where the plant is not uncommon." This, if correct, means that the specimen was collected in 1827–1831. These data indicate the probability that citrus canker originated in India and Java rather than in other regions of the Orient.

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CONTROL OF OAT SMUT[1]

V. H. YOUNG AND C. K. MCCLELLAND

(Accepted for publication Oct. 26, 1932)

The control of the smuts of oats by the use of formaldehyde solutions applied in a variety of ways is well established. When properly carried out, treatment with formaldehyde has given excellent control of both the loose and covered smut of oats. Recently, several workers have reported efficient control of these smuts by the use of organic-mercury dusts and by the use of formaldehyde adsorbed on some inert carrier. Karns[2] also has reported that elemental iodine, dissolved in carbon bisulphide, is efficient for this purpose.

Experimental tests in 1931 and 1932, at the University Farm, Fayetteville, Arkansas, of some of these newer oat-smut treatments gave rather striking results, which are here reported. Seed of Fulghum oats obtained in 1930 from a badly smutted plot on the university farm was used in these experiments in 1931 without the addition of further inoculum. Seed from the nontreated checks of 1931 was used in 1932. The following dusts were applied in 1931: Ceresan, active principle 2 per cent ethyl mercury chloride; Dubay No. 952 K, active principle ethyl mercury tartrate 2 per cent; Dubay No. 952 C, active principle ethyl mercury tartrate 1 per cent; Dubay No. 500 LL, active principle ethyl mercury arsenate 1 per cent; Merko, the active ingredient of which is said to be an inorganic mercury compound; Corona Oat Dust, said to be activated formaldehyde; and Corona No. 174 E, said to contain a soluble organic mercury compound. In addition to the dust treatments, seed for one plot in 1931 and for 3 plots in 1932 was treated with a 10 per cent solution of iodine in carbon bisulphide at the rate of ½ oz. of elemental iodine per bushel. In 1932 Dubay No. 1134, active principle ethyl mercury phosphate 2.5 per cent, and the liquid-formaldehyde treatment[3] were added to the tests. Merko, which failed to control oat smut in 1931, and Corona No. 174 E, which was less effective than the other dusts employed in 1931, were discarded.

Dusts were applied by means of a barrel churn to which a baffle plate had been added at one end to secure more thorough mixing. Each batch

[1] Contribution from the Departments of Plant Pathology and Agronomy. Published with the approval of the Director of the Arkansas Agricultural Experiment Station as No. 322, of the University of Arkansas Journal Series.

[2] Karns, George M. Effectiveness of iodine in the control of smut on oats. Ind. & Eng. Chem. 22: 864. 1930.

[3] Formaldehyde was applied by the spray method as described by Tapke, V. F. Formaldehyde seed treatment for oat smuts. U. S. Dept. Agr. Misc. Pub. No. 21. 1928.

of seed was rotated for 5 minutes and the interior of the churn was washed out and dried between treatments. Dusts were applied at the rate of 3 oz. per bu. except Dubay No. 1134, which was employed at the rate of 1 oz. per bu., according to the recommendation of the manufacturers. The iodine in carbon bisulphide solution was added to the oats in a glass fruit jar and the oats were then shaken until uniformly discolored by the solution. They were then spread out in a thin layer to allow evaporation of the carbon bisulphide.

Treated oats were planted in 1931 in $\frac{1}{10}$-acre plots on land that had been in corn for the 3 preceding years, each treatment being repeated 3 times with the exception of the iodine in carbon bisulphide treatment, of which there was only 1 plot. Seven nontreated checks were used, the last in the first and second series appearing as the first in the series following. All seed for the 1931 tests was sown March 18, 1931, 2 days after treating. Soil moisture and temperature conditions remained favorable for the growth of oats throughout their growing period. Stand counts and counts of infected heads were made shortly before harvest, when all heads had appeared. By means of a wire hoop, 22 in. in diameter, 10 samples were taken at random from each plot. In certain cases, though these counts indicated that no smut was present, a careful survey of the plots revealed the presence of a very small number of smutted panicles. These were equivalent to a mere fraction of one per cent of the total number of panicles in the plot. A thorough search of all plots indicated that loose smut (*Ustilago avenae*) was the predominating form, both in 1931 and 1932.

The dust treatments used, the percentage of infection in each plot, the average percentage of infection for each treatment, the threshing percentage, yields in bushels per acre, and the gains or losses in bushels per acre are given in table 1. Theoretical yields of interpolated checks have been used as a basis for computing gain or loss from treatments over checks, and gains or losses without adjustment also are shown with percentage values.

A statistical interpretation of the yield data given in table 1 shows that the standard deviation of an individual plot yield as determined by the check-plot method is 11.66 bu. or 31.3 per cent. The standard deviation of the mean for 3 plots is 6.74 bu. The difference between plots necessary for significance is $2\sqrt{2}$ 6.74 or 19 bu., assuming that the treated plots are subject to the same error in bushels as the nontreated ones. According to this standard, no significant differences in yield exist, due to the high variability as shown by the check plots. However, the reduction in the incidence of smut resulting from all treatments except Merko, which the manufacturers do not recommend for oats, is clear-cut and, on the whole, satisfactory. As shown in table 1, there seems to be little relation between the treatments

TABLE 1.—*Effect of dust treatments on the incidence of smut on Fulghum oats and on yield at University Farm, Fayetteville, Arkansas, 1931*

Treatment / Series	Percentage of infection from counts of 10 areas 22″ in diameter				Threshing percentage	Yields per acre				Gains		
	A	B	C	Averages		A Bu.	B Bu.	C Bu.	Aver. yield Bu.	Bu.	Bu.[a]	Per cent
Check	15.92	20.46	20.11	20.08	50.67	59.2	34.8	26.3	37.3			
	19.96	22.24	23.51			42.7	24.8	35.9				
	20.46	20.11	18.36			34.8	26.3	50.6				
Dubay No. 952 K	.09	0	0	.03	57.02	53.9	35.6	28.1	39.9	0.94	2.6	7.0
Ceresan	0	0	0	0	55.54	50.9	37.8	34.7	41.2	3.31	3.9	10.5
Dubay No. 500 LL	0	.96	.52	.49	54.54	48.0	40.5	38.8	42.4	5.68	5.1	13.7
Corona No. 174 E.	3.02	2.37	2.91	2.77	56.48	52.7	35.5	40.6	42.9	7.32	5.6	15.1
Merko	18.94	20.32	19.89	19.72	54.60	37.3	36.7	34.4	36.2	0.98	−1.1	−3.0
Dubay No. 952 C	0	0	0	0	53.37	44.1	41.7	55.3	47.0	11.17	9.7	26.1
Corona Oat Dust	0	0	0	0	55.04	40.9	38.9	55.3	45.1	8.5	7.8	21.0

[a] These gains obtained without adjustment.

and the ratio of grain to straw as expressed by the threshing percentage,[4] and no relation between treatments and stand could be noted.

The 1932 tests were planted March 19, 1932. Unusually dry weather during the spring months resulted in short straw and a low yield of grain. The dust applications were made as in the previous year and 3 plots from each lot of treated seed were again planted. All seed was sown as soon as treated, with the exception of that treated with formaldehyde and that treated with iodine, which was allowed to stand 24 hours before planting. Five check plots were included. As mentioned previously, the seed used in 1932 was obtained from one of the check plots of the 1931 tests in which about 20 per cent of the panicles were infected. Five areas on each plot and then 5 duplicate areas on the 5 check plots were counted and percentages of infection calculated.

Table 2 gives the results of the counts as percentage of infection and also the yields of the various plots in bushels per acre.

As will be seen from table 2, the percentage of infection on plots where nontreated seed was used was 19.25 per cent, which was not markedly different from that of the previous year. All of the treatments gave good control of smut except the iodine treatment, which was not nearly so effective as in 1931, possibly because thorough coverage was not secured. The dust treatments were all effective, several of them showing perfect control.

On the plots having treated seed there was a consistent, though not large, gain in yield, varying from 2.7 to 6.8 bu., or 12.9 to 32.5 per cent. These figures are from nonadjusted yields; no interpolations between check plots were made because, due to amount of seed available, the plots varied in size. The gains averaged 4.3 bu. or 20.55 per cent on the plots where dust-treated seed was used in contrast to a gain of only 17.7 per cent for the liquid-formaldehyde treatment.

A statistical treatment of the yield data shows that the standard deviation of a single yield was 2.16 bu. or 10.3 per cent, showing the variability to be only one-third as much as in 1931. The standard deviation of three plots is 1.25 bu. Again using twice the square root of 2 multiplied by the standard deviation of 3 plots as a measure of significance, we have 3.5 bu., the magnitude of a difference needed to establish significance. All treatments except Dubay 1134 and iodine may be taken as having given significant differences in yield. The increased yields of certain of the dust treatments over the liquid formaldehyde treatment suggest the possibility of stimulatory effects due to the treatment in addition to increases from the control of oat smut, such as have been noted recently by workers in other

[4] The threshing percentage represents the relation between the weight of threshed grain and the weight of bundles.

TABLE 2.—*Effect of dust treatments, liquid formaldehyde, and iodine in carbon bisulphide on the incidence of smut on Fulghum oats and on yield at University Farm, Fayetteville, Arkansas, 1932*

Treatment	Percentage of infection from counts of 5 areas 22″ in diameter						Yield per acre				Gains	
Series	A	B	C			Averages	A Bu.	B Bu.	C Bu.	Aver. yield in bu.	Bu.	Per cent
No treatment	21.6	18.2	27.9	20.	14.7		23.5	23.3	20.1			
dupl. 5 counts	17.3	20.8	17.5	20.5	14.	19.25	18.6	18.8		20.9		
Dubay No. 1134	0	0	.4			.13	27.8	21.0	22.2	23.7	2.8	13.4
Dubay No. 500 LL	0	1.3	.4			.57	24.8	24.8	23.5	24.4	3.5	16.7
Dubay No. 952 K	0	0	0			0	25.5	23.1	23.7	24.1	3.2	15.3
Dubay No. 952 C	0	0	0			0	28.	26.1	29.1	27.7	6.8	32.5
Ceresan	0	0	0			0	28.5	23.5	25.1	25.7	4.8	23.
Corona Oat Dust	0	0	0			0	25.5	22.3	29.0	25.6	4.7	22.5
Formaldehyde	0	.9	0			.3	24.0	23.3	26.4	24.6	3.7	17.7
Iodine	5.	4.8	5.2			4.65	21.2	24.8	24.9	23.6	2.7	12.9
dupl.		3.6										

States. Sufficient data, however, are not available to draw definite conclusions.

The results of the iodine-carbon bisulphide treatment of oats are not included in table 1, as only one plot was used in 1931. Counts of 10 areas selected at random, as in the case of the other plots, gave no smut whatsoever in the iodine plot, and even a careful search of the whole plot revealed the presence of only the slightest trace of smut. The yield per acre computed from the iodine plot was exceptionally high, but it is believed that soil differences may be responsible for part of this increase. In 1932 iodine gave much poorer control than did the other treatments, and the increase in yield, when tested statistically, did not appear significant.

SUMMARY

Certain dust treatments, employed for 2 years, gave excellent control of loose smut of oats, whereas others gave only a fair degree of control or were ineffective. In 1932 the dust treatments compared favorably with the liquid-formaldehyde treatment.

Iodine, dissolved in 10 per cent solution in carbon bisulphide, applied at the rate of ½ oz. per bu., gave nearly perfect control of loose smut of oats in 1931 but was much less effective in 1932.

None of the treatments employed appeared to affect the stand or to have any marked effect on the ratio of straw to grain as expressed in the threshing percentage.

Variability in the check plot yields in 1931 was so great that significant statistical differences were not made. In 1932 less variability was shown and the gains, for the most part, attained significance.

ARKANSAS AGRICULTURAL EXPERIMENT STATION,
FAYETTEVILLE, ARKANSAS.

ROGUING AS A MEANS OF CONTROL OF TOBACCO MOSAIC

FREDERICK A. WOLF[1]

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It has long been known that mosaic-infected plants in the field serve as sources of inoculum for near-by healthy plants. As long ago as 1904, Selby[2] suggested the prompt removal, from fields, of diseased plants when they were noted early in the growing season. More recently Valleau and Johnson[3] regard this practice as advisable. Apparently, however, no experimental data are available on the effectiveness of this procedure. Accordingly, the writer undertook to test the efficiency of roguing as a field practice in mosaic control.

The fields used for these trials were set with healthy plants, as determined by successive examinations of the seed beds. These examinations were made at intervals, beginning prior to transplanting and extending through the transplanting season. The selected fields had borne a mosaic-affected crop during the previous year, and their soils were, in consequence, infested. The writer's observations indicate that, even though fields are set with mosaic-free seedlings, mosaic invariably develops, provided such fields have produced a diseased crop during the preceding season. Infested soil, in such cases, constitutes the initial source of inoculum. Moreover, these observations show that when infested fields are examined as soon as the plants have become established, following transplanting, they will be found to contain an average of less than one per cent of affected plants. These plants then serve as sources for the increase of the disease.

The removal of mosaic plants, as early as possible, was the procedure employed in the present trials with the results summarized in the following tabulation.

It may be noted, first of all, that roguing did not result in eradicating mosaic except in fields 1 and 8. Among a total of 48,013 plants in these 13 fields, 177 diseased ones were destroyed, and at the beginning of harvest 162 mosaic ones remained. When the first examination was made some of the inoculated plants had been transplanted for too short a period to allow sufficient time for the development of symptoms. Such plants were plainly mosaic at the date of second inspection. The removal of diseased plants at these 2 inspections should have resulted in the complete eradication of

[1] Pathologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] SELBY, A. D. Tobacco diseases and tobacco breeding. Ohio Agr. Exp. Sta. Bul. 156: 87–114. 1904.

[3] VALLEAU, W. D., and E. M. JOHNSON. Observations and experiments on the control of true tobacco mosaic. Ky. Agr. Exp. Sta. Bul. 280. 1927.

TABLE 1.—*Results of roguing of mosaic plants as a means of control*

Year	Field No.	Number of plants rogued	Date of removal from field	Total number of plants removed	Number of plants remaining at maturity of crop	Total number of plants in the field
1928	1	2	June 18	2	0	4,050
1929	2	18 2	June 8 " 21	20	3	2,813
"	3	11 5	June 25 July 5	16	7	1,560
"	4	5 15	June 25 July 1	20	9	2,585
"	5	11 3	June 19 July 1	14	3	3,440
"	6	2 8	June 19 July 1	10	8	1,095
"	7	6 12	June 17 " 29	18	10	4,080
"	8	1 1 2	June 17 " 29 July 9	4	0	5,600
"	9	11 4	June 25 July 5	15	7	1,560
1930	10	10 4	June 6 " 18	14	52	3,050
"	11	3 2	June 5 " 19	5	5	8,520
"	12	2 9	June 21 July 1	11	5	2,160
"	13	10 6 12	May 28 June 5 " 19	28	53	7,500
Total				177	162	48,013

mosaic from all fields. By the time that the crop was ready to harvest, however, the growers had introduced mosaic anew from near-by affected crops, into all except fields 1 and 8. This explanation is supported by the fact that the tops and suckers, instead of the entire plants, were mosaic, and that the crops in adjoining fields were diseased.

It would have been interesting to have known what proportion of the total number of plants in these 13 fields would have become mosaic by

harvest time if roguing had not been employed. Since it was impossible to do this or to have entirely satisfactory check experiments in adjoining parts of the same fields, comparisons must be made in another way. Abundant data are at hand for such comparisons, since records have been made, over a 3-year period, of the number of mosaic plants in 125 fields in which infested soil constituted the source of inoculum. The average initial infection in 12 representative fields of this type was 0.7 per cent, and by harvest time an average of 13.5 per cent had been diseased. With this as a basis for comparison, 336 plants in the 13 fields should have been mosaic at first, and by harvest time this number should have increased to 6,481. The actual presence of 339 plants, approximately half of which were a total loss because the plants were removed, instead of an expected 6,481, among a population of 48,013, constitutes very satisfactory control.

No effort was made to replant with mosaic-free seedlings in these 13 fields to replace the 177 plants that were removed. The resetting of healthy plants in place of mosaic ones cannot be recommended as a general practice, since the experience of all who have attempted to do this shows that a considerable proportion become inoculated by being set where diseased ones have been removed. This is supported by the results in the following case in which approximately 98 per cent of the plants in a particular field were found to be infected when the crop was cultivated for the first time. Accordingly, all of the plants from a portion of this field were removed and the area was reset with healthy plants. Twenty-seven days later 16 per cent were mosaic, 13 days thereafter the number had increased to 25.5 per cent, and after an additional 11 days 57.1 per cent were affected.

Attention should also be called to the fact that the roguing of crops set with plants from infested seed beds was not attempted. In such cases, experience has shown that the initial infection is too great to permit such a procedure to be profitable since upwards of 10 per cent of the plants are visibly diseased as soon as they have become established in the field.

SUMMARY

When healthy plants are set in fields which produced a mosaic crop during the preceding season, normally only a small proportion of the plants are infected at time of first cultivation. Under these conditions, roguing early in the season is an effective check on the spread of mosaic.

Rouging of fields set with seedlings from infested seed beds is not advisable, because it necessitates the removal of too large a proportion.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA.

EFFECT OF MOSAIC OF FLUE-CURED TOBACCO ON YIELD AND QUALITY

FREDERICK A. WOLF[1] AND E. G. MOSS[2]

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Most growers of flue-cured tobacco are of the opinion that mosaic causes losses through decrease in yield and impairment of quality. Some maintain, however, that it does no damage. Within the past few years the results of several investigations on this phase of the mosaic problem with other types of tobacco have been published. Valleau and Johnson[3] found that mosaic reduced the yield of Burley tobacco approximately one-third and the value two-fifths when all plants were inoculated at time of transplanting. When inoculation was delayed until topping time, there was no decrease in yield, but there was a decrease of one-fourth in crop value. With Maryland tobacco, McMurtrey[4] found that the average reduction in yield for a 3-year period was 30 to 35 per cent, and in value, 55 per cent when all plants were inoculated at time of transplanting. The damage was almost as great as this when the plants were inoculated a month after transplanting time. There was no significant reduction in yield, however, when inoculation was delayed until time for topping; yet, even at this stage, mosaic adversely affected the quality.

Since there are no available data on the effect of mosaic on yield and quality of flue-cured tobacco, such studies were undertaken in 1929 and 1930 at the Tobacco Experiment Station, Oxford, N. C. Four adjacent plots of $\frac{3}{8}$ acre each were set with tobacco. All were planted on the same day, were given equal applications of fertilizer, and cultivated alike. The first was inoculated when the plants were set, the second a month later, the third at time of topping, and the fourth was left uninoculated as a basis for comparison. The inoculum was prepared by crushing with the fingers the leaves of a green mosaic plant and suspending the macerated material in a small quantity of water. The tops of the seedlings were dipped into this inoculum just prior to transplanting, in the case of those that were inoculated when they were transplanted. The inoculum, simi-

[1] Pathologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] Agronomist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture and Assistant Director, Tobacco Branch Station, North Carolina Department of Agriculture.

[3] Valleau, W. D., and E. M. Johnson. The effect of a strain of tobacco mosaic on the yield and quality of Burley tobacco. Phytopath. 17: 523–527. 1927.

[4] McMurtrey, J. E., Jr. Effect of mosaic disease on yield and quality of tobacco. Jour. Agr. Res. 38: 257–267. 1929.

larly prepared, was communicated to the block of plants inoculated a month later, and to those inoculated at topping time by wetting the fingers with inoculum, and then gently rubbing, between the thumb and fingers, the bud leaves. Nearly all plants inoculated developed evident symptoms within 2 weeks. Those inoculated at time of transplanting remained stunted during the first portion of the growing season, but were normal in size by the end of the season. Moreover, they matured about 2 weeks later than the plants on the noninoculated plot.

The results of these tests for the season 1929 and 1930 are presented in the following table.

TABLE 1.—*Influence of mosaic on yield and quality of flue-cured tobacco*

Plot No.	Year	Time of inoculation	Yield per acre	Decrease in yield	Acre value	Decrease in acre value
			Pounds	*Per cent*		*Per cent*
1	1929	At transplanting	668	23.2	166.22	42.3
	1930	do	642	39.6	29.24	66.8
Average			655.0	31.4		54.6
2	1929	One month after transplanting	646	25.7	153.42	47.2
	1930	do	739	34.6	57.46	37.0
Average			692.5	30.1		42.1
3	1929	At topping	734	15.6	208.00	23.6
	1930	do	917	18.8	79.37	23.9
Average			825.5	17.2		23.8
4	1929	Control, not mosaic	870		272.59	
	1930	do	1,130		91.24	
Average			1,000			

It is evident that mosaic adversely affects both the yield and quality of flue-cured tobacco, dependent upon the stage of growth at which infection occurs. The 2-year average yield on the mosaic-free plot was 1,000 pounds; that on the plot inoculated at time of setting the plants was 655 pounds, or a decrease in yield of 31.4 per cent and a decrease in value of 54.6 per cent. The yield on the plot inoculated approximately a month after transplanting was 692 pounds, a reduction in yield of 30.1 per cent and in value of 42.1 per cent. The plot inoculated at time of topping yielded 825 pounds, a decrease in yield of 17.2 per cent and in value of 23.8 per cent.

Only in rare cases, when judged by the writers' observations, which have extended over three seasons, is mosaic, in any grower's crop, so destructive as in plots 1 and 2. However, an average of approximately 20 per cent of the total crop may be mosaic at time of topping. The loss occasioned by this proportion, plus that resulting from the wholesale inoculation that occurs during the operation of topping, should result in a decrease in yield and value in excess of that shown in plot 3. At any rate, these data show the necessity for preventing infection and for the employment of cultural practices that will reduce the spread of the disease.

SUMMARY

Mosaic seriously reduces the yield and impairs the market value of flue-cured tobacco. The degree of damage is related to stage of growth at which infection occurs, for, when infection occurs early in the growth of the crop, it occasions the greater losses.

PHYTOPATHOLOGICAL NOTES

Downy Mildew of Tobacco on Pepper, Tomato, and Eggplant.—Downy mildew of tobacco (*Peronospora hyoscyami,* de By.) was first widely prevalent in the tobacco area of eastern South Carolina in 1932. Soon after the tobacco plants in the beds became diseased, reports were received that pepper plants in beds also were diseased. The growers were convinced that the disease of pepper was caused by the downy mildew or blue-mold fungus because of the similarity of effects on the plants. This belief was strengthened by the fact that in the 30 years of culture of the Cayenne type of peppers in the vicinity of Florence, South Carolina, no serious difficulty had been encountered in growing the plants in beds prior to the appearance of the tobacco disease.

A Peronospora was found on some pepper plants of one lot sent to the senior writer, while it was not found on the plants of another shipment. Plants later collected by the writers showed the same fungus but it was not abundant. Young pepper plants were killed when attacked by the fungus in 1932. Some plant beds were seen with nearly every plant destroyed.

The first diseased pepper plants were noted on April 21, 1933, in a row at the end of an infected tobacco-plant bed, and diseased plants in several pepper beds were observed within the following 3 days. A Peronospora was found on about one-third of the diseased leaves. If infection occurred when the plants were almost large enough to be set in the field, as was generally the case in 1933, the infected leaves were shed, but few plants were killed in contrast to the heavy mortality of young plants in 1932. This article may be of interest at this time because of the recent reports[1] that a fungus apparently identical with *Peronospora hyoscyami* has been found on pepper and also on tomato.

On April 24, 1933, 2 sq. yd. of a pepper-plant bed were used in a cross inoculation of *Peronospora hyoscyami* from tobacco to pepper. An adjoining noninoculated area of the same size was used as a check. The beds were watered and the plants then covered with a mist of water from a small hand sprayer. Tobacco leaves showing a dense coating of fresh spores were shaken above and then dragged over the plants in one bed. A muslin cloth was then placed over the inoculated and noninoculated beds and moistened at intervals of approximately 1½ hours during the daylight hours of the 2 following days. Three days following inoculation, a few diseased plants were seen scattered throughout the large bed, a part of which was being used for the experiment. Infection conditions were thence unfavorable and,

[1] Plant Disease Reporter 17: 37, 1933.

fortunately, there was no further spread of natural infection. At the end of 7½ days, 85 per cent of the plants in the inoculated area showed definite symptoms of the disease, while only about one per cent of those in the check area showed the disease.

Eight sweet pepper plants of an unknown variety growing in pots were inoculated from infected tobacco plants on May 6, 1933. Four pots of plants used as checks were not inoculated. Definite symptoms of disease were evident on the plants in 3 pots of the inoculated group 5½ days later and a plant in a fourth pot was found diseased on the eighth day after the inoculation was made. The noninoculated plants remained healthy.

An attempt was made to infect tobacco plants with the fungus from pepper; within 42 hours after inoculation, practically all of the plants in both the inoculated and noninoculated areas were showing an abundance of Peronospora spores and sporophores on the under surface of the leaves. This was accepted as rather conclusive evidence that natural infections had occurred before the inoculations were made. No further inoculations from pepper to tobacco could be made, since all tobacco plants at the Experiment Station became diseased a few days after the first inoculation.

A Peronospora apparently identical with that on tobacco has been found by the writers on tomatoes as reported by others[2] and also on a new host,

TABLE 1.—*Frequency distribution of spore size of a Peronospora on tobacco, tomato, pepper, and eggplant*[a]

Classes μ	Length				Width			
	Tobacco	Tomato	Pepper	Eggplant	Tobacco	Tomato	Pepper	Eggplant
8.1							1	1
10.8–12.2	2		1		21	51	15	234
12.4–13.9	2	3	2	8	127	207	223	278
14.4–15.1	5	5	9	27	116	147	149	60
15.3–16.3	81	128	60	194	266	182	194	25
17.2–18.2	103	109	75	127	43	8	13	0
18.6–19.8	180	197	209	168	22	5	5	1
20.1–21.1	94	74	84	42	4			1
21.7–22.9	98	74	124	26	1			
23.0–24.4	25	9	34	7				
24.9–25.7	3	1	2	1				
27.1	7							
Total	600	600	600	600	600	600	600	600

[a] Average size of spores on tobacco 19.2 × 15.4 μ; tomato 18.6 × 14.6 μ; pepper 19.5 × 14.9 μ; eggplant 17.9 × 13.1 μ.

[2] Plant Disease Reporter. See footnote 1.

the eggplant. The tomatoes and eggplants were growing in a bed a few feet away from tobacco beds.

Measurements of 600 spores each from tobacco, pepper, tomato, and eggplant have been made and the results are presented in table 1. Similarity in spore size is evident.

The appearance of a distinctly new disease of pepper, tomato, and eggplant since the recent introduction of *Peronospora hyoscyami* on tobacco, the close association of the diseased plants with infected tobacco plants, and the apparent morphological similarity of sporophores and spores, seem to indicate that *P. hyoscyami* can attack these plants. The foregoing facts together with the successful infection of pepper plants on two occasions with spores from tobacco seem to be fairly certain evidence that *P. hyoscyami* will infect pepper.—G. M. ARMSTRONG AND W. B. ALBERT, South Carolina Experiment Station, Clemson College, South Carolina.

Manufactured Tobacco, a Source of Inoculum for Mosaic in Flue-cured Tobacco.[1]—Consideration has been given, since the spring of 1928, to the source of mosaic virus in seed beds in North Carolina. Observations on the time of first appearance of tobacco mosaic in the beds, on its peculiar distribution therein, and on the presence of visible symptoms on the youngest leaves of seedlings whose older leaves are apparently normal lead one to conclude that in many cases the virus is introduced by the grower through the use of various forms of manufactured tobacco. Manifestly, such epiphytological evidence does not constitute unequivocal proof. The following studies were made, therefore, for the purpose of determining whether, by direct experimentation, it could be found that manufactured tobacco is a source of mosaic virus, as has been reported by Valleau and Johnson[2] and by Fukushi.[3] Since manufactured tobacco is apparently the most important source of inoculum for mosaic in seed beds in flue-cured tobacco districts, a fact not generally known, the publication of the present report, which confirms the findings of other investigators and is supported by a considerable body of unpublished epiphytological data, seems to be warranted.

Two lines of evidence were followed in testing the infectiousness of tobacco. The first involved the use of air-cured mosaic-affected leaves, and the second of different forms of manufactured tobacco procured from retail stores.

[1] This report, prepared by the junior writer, is based upon studies by Hans J. Busch, Berlin-Spandau, Germany, during his residence as a graduate student at Duke University.

[2] VALLEAU, W. D., and E. M. JOHNSON. Commercial tobaccos and cured leaf as a source of mosaic disease in tobacco. Phytopath. 17: 513–522. 1927.

[3] FUKUSHI, T. On the modes of transmission of the mosaic disease of tobacco. Jour. Sapporo Soc. Agr. & For. 22: 305–320. 1931.

Mosaic-affected leaves were collected in August, 1928, were then air-cured, stored in cans, and through the courtesy of a manufacturer, were converted into chewing tobacco during April, 1929. This tobacco was employed in the spring and summer of 1929 in making a series of inoculations. It was applied to healthy plants either as spittle or was sprinkled upon them. In the latter case the inoculum was prepared by soaking a small quantity of tobacco in water. Rapidly growing potted plants, when inoculated, contracted mosaic within a period of about 2 weeks. Although all tobacco grown in the Coastal Plain and Piedmont sections of North Carolina is flue-cured, and the temperature reached during the last stages of curing usually destroys the virus, yet the infective agent occasionally survives the curing process. Even if it were destroyed in all flue-cured tobacco, manufactured tobacco might still remain a source of inoculum, because all brands of granulated, plug, and cigarette tobaccos are blended and, consequently contain air-cured tobacco grown in sections where mosaic is annually a more or less generally prevalent disease.

The studies bearing on the infectiousness of 45 brands of manufactured tobacco involved an examination of 11 brands of snuff, 17 of chewing tobacco, 10 of granulated pipe tobacco, and 7 of cigarettes. Duplicate samples of 15, and triplicate samples of 4 of the brands were tested, making a total of 68 samples. The samples were placed in flasks of sterilized water, and there allowed to soak for about 12 hours. This material was then used as an inoculum. Sterilized cotton swabs on sticks were dipped into the inoculum and the latter was gently brushed upon the buds of healthy plants. Three plants were inoculated with each sample. Sterile swabs were thus employed with each sample. This method has the additional advantage of preventing accidental inoculations, since the plants were not touched directly by the operator. After 12 to 18 days some of the plants inoculated with 2 of the brands of chewing tobacco and 2 of snuff showed symptoms of mosaic, while those inoculated with the remaining 41 brands remained mosaic free.

Infectiousness was verified in each case by testing another sample from the same package. In some cases only one of the 3 inoculated plants became mosaic. A similar failure of all inoculated plants to develop symptoms was reported by Valleau and Johnson.[4] In some cases one sample of a particular brand contained the virus, while another gave no infection. At least it may be concluded from these experiments that occasional samples of certain brands of manufactured tobacco contain the mosaic virus. These results support those of Valleau and Johnson,[4] who found among manufactured tobaccos 4 brands of plug, 7 of cigarettes, and 5 of granulated

[4] *l. c.*

tobacco that were infectious. They also support the more recent results of Fukushi[5] who reported that 25 among 30 packages of 4 brands of pipe tobacco were infectious. He inoculated plants by rubbing the pipe tobacco between the fingers and then, with contaminated fingers, slightly crushing the leaves.

It may be noted that in the present studies cigarettes and granulated smoking tobaccos were not found to be infectious. Valleau and Johnson's[6] studies led them to conclude that these forms of tobaccos are of minor importance in the mosaic problem.

When consideration is given to the readiness with which virus is spread, it is apparent that the presence of mosaic in the seed beds is an exceedingly important matter, even though only a few plants are affected. This fact is scarcely appreciated except by those who have had experience with tobacco mosaic.—HANS J. BUSCH AND FREDERICK A. WOLF.

Correction in Authority for Cronartium ribicola.—As pointed out by Sydow,[1] and independently confirmed by the writer, *Uredo ribicola* Lasch does not belong in the genus Cronartium, as heretofore assumed by the writers[2] and others. This fact requires the correct citation to be *Cronartium ribicola* Fischer, according to taxonomic procedure under the International Rules of Nomenclature. A full statement of the situation will probably be made by Sydow in the Annales Mycologici.—J. C. ARTHUR, Lafayette, Indiana.

[5] *l. c.*

[6] *l. c.*

[1] SYDOW, P. & H. Monographia Uredinearum 4: 590. 1924.

[2] ARTHUR, J. C. Phytopathology 23: 559. 1933.

ANNOUNCEMENT

The session at Boston, December 28 to December 30, 1933, will be the 25th annual meeting of The American Phytopathological Society. In recognition of this event it is hoped that the president, Doctor Arthur, dean of American mycologists and phytopathologists, will speak briefly on progress in Phytopathology during this period and on some of the great personalities in this field.

The Society will hold joint sessions with: (1) Section G and other Botanical Societies, Thursday afternoon; (2) the Mycological Society, Friday; and (3) the Potato Association of America, Saturday. On Thursday afternoon the annual conference on extension work in plant pathology will be held.

Our headquarters hotel, the Westminster, has some very attractive suites for 3 to 6 persons. To assist the managers in arranging accommodations for the December meeting, it is suggested that members write the hotel at once concerning reservations, informing them of instances where groups would prefer to be located together or in near-by rooms, or of desire on the part of two individuals to share a double room.

Scientific sessions will be held in the Biological Laboratories, Harvard University, where there will be space for exhibits of slides, charts, and other material related to papers presented. Packages containing material for exhibition should be marked Phytopathological Exhibit and be mailed or expressed to Dr. W. H. Weston, Jr., Biological Laboratories, Divinity Avenue, Cambridge, Massachusetts.

Business meetings will be held as usual on the mornings of Thursday and Saturday.

The annual dinner of the Society will be Thursday evening. While it is not possible at this time to announce either the participants or their subjects, it seems certain that the program for this classic event will be in line with the best traditions of the Society.

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ETIOLOGICAL PHYTOPATHOLOGY

GEORGE K. K. LINK

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I. CONCEPTS, SCOPE, AND RÔLE

Like other experimental sciences, phytopathology aims to establish by experiment, that is, by deliberate change of one factor at a time, the simultaneous and serial antecedents out of which a pathic event (12) arises at a given time and place within an affected plant. It aims to analyze into their constituent part events complex total pathic events, such as an infectious disease, that occur during or follow development, and to establish the factors out of which each part event in the series arises. In so far as these factors are antecedent to, *i.e.*, condition and/or incite, pathic events, they are pathogenic factors (12). In this process of analysis or detailed description, stimulus-reaction chains or chains of causation are established in which the initial stimulus and the final reaction often are separated temporally and spatially by a long series of intermediate reactions and stimuli. When all of the series of causation chains of an event and their interrelations have been completely established, each part event, and, consequently, the complex total event, is said to be causally explained. In practice a causal explanation merely means that attention has been focused upon one part of the complex, which is called the effect, and then upon a striking feature of the complex of factors antecedent to it, which is called the cause.[1]

The branch of plant pathology that concerns itself with the causal analysis of pathic events has long been designated as etiology or etiological pathology. The term etiology[2] is derived from aitia (αἰτία), which means

[1] According to the present conception of the principle of causality, experiment is not able to establish absolute, invariant relations between so-called antecedents and consequents. Absolute predictability of events consequently is no longer considered the goal or even the ideal of science. Instead, science aims to establish by experiment the probabilities of apparent relations of events. While this conception is not a new one in the history of science, the advent of the new experimental and theoretical physics has been largely instrumental in bringing it into prominence.

[2] Democritus (5th century B. C.) was the first to use the term etiology. While it and its adjective, etiological, do not appear in the only treatise on the healthy and pathic behavior of plants that has come down to us from Greek antiquity, the term *aitia* is used throughout this discourse and in its title. This inquiry (27) by Theophrastus

blame or cause, and from logia (λογία), which means a reasoned discourse, an inquiry. Consequently, etiology is a system of knowledge or an inquiry dealing with the antecedents of events and with the rational study of the concepts in which the relations of antecedent and consequent are expressed.

By a shift, the term etiology has come also to mean the totality of the antecedents of an event, rather than the body of knowledge pertaining to these antecedents. The adjective etiological has experienced a similar shift in meaning. Thus the phrase "the etiology of mosaic" when used in this sense means the complex of antecedents out of which mosaic arises.

By a further shift the term etiology has been restricted to the obvious external antecedent of a pathic event, or to the body of knowledge dealing with this obvious causal factor. This factor is often designated as *the cause,* with an implication that it is the *sole cause* (12). In many text-books and special treatises the category of etiology includes only the specific pathogenic microorganism when infectious diseases are discussed, or the obvious external factor when noninfectious diseases are the object of inquiry. The specific pathogenic microorganism is often designated as *the pathogen.*

(370–286 B. C.) bears the title (ABOUT THE) CAUSES OF PLANTS (*Peri Phyton Aition*). Etiological pathology therefore can trace its lineage to the "Father of Botany."

By the end of the 2nd century A. D. the terms etiology and etiological had begun to have technical meanings. Galen (130–200 A. D.) in his classification of the branches of medicine (11) stated: "The etiological, which is also called pathological, is that part (of medicine) in which we examine that which happens against nature and investigate the causes of disease and the concourses of symptoms and inquire closely into the conditions of affections." Incidentally, this is Galen's definition of the field of pathology. While Galen defined the field of the pathological, he did not introduce the term pathology. Pathology had not yet been recognized as a distinct science. The word is not found in Greek lexica. Its Latin form, *pathologia,* had made its appearance by the 6th century A. D. and occurs for the first time in Corpus Glossariorum Latinorum (ed. Götz) IV, 263, 3; V, 384, 47, and is defined as *ratio passionis,* a reasoned discourse on affections. According to Galen, pathology is that part of medicine that concerns itself with the causal analysis of the subject matter of medicine. This still is the current definition of the field of human pathology. Phytopathology, historically and currently, is the botanical equivalent of medicine and hygiene and includes not only etiology but diagnosis (symptomatology) and preventive and remedial control (plant protection) as well.

Other biological sciences, such as physiology and genetics, although engaged in description of the probable sequences of relations of their subject matter, no longer recognize the etiological approach by a distinctive term. As theoretical and experimental phytopathology becomes a mature natural science, it too will become so thoroughly etiological that it probably will cease to designate its etiological descriptions by a differentiating term. Hence, the terms etiology and etiological probably will disappear from the vocabulary of theoretical pathology just as they have been dropped from the vocabularies of physics, chemistry, and physiology.

This highly limited and artificial conception of etiology and the fact that any scientific analysis that is more than a description of the static aspects of events concerns itself with conceptual and experimental analysis of the antecedents of events have been important factors in focusing the attention of phytopathologists upon pathogenic bacteria and fungi in the causal analysis of plant diseases. As a consequence, phytopathological activity and literature have been so largely concerned with the taxonomy, morphology, cytology, physiology, ecology, and genetics of pathogenic microorganisms, especially fungi, that phytopathology now is regarded as essentially parasitological mycology.

Recently, Morstatt (14) and Whetzel (26) have protested against the dominance of etiology in phytopathology. Morstatt contends that the etiological point of view stands in the way of a thoroughgoing general phytopathology because it has forced the pathic plant with its disturbed structures and processes out of the center of interest and consideration and thereby arrested the development of cellular phytopathology, which he considers the basis for a general phytopathology. He asserts further that the dominance of etiology has split the field of phytopathology into an applied botany which is restricted to applied mycology, and into an applied zoology, which is limited to applied entomology (13), and thereby retarded development of a general phytopathology. Finally, he contends that this preoccupation with eitology has led to neglect of those ecological factors, other than the parasite, of the pathic plant that play a rôle in parasitic diseases and to an almost complete disregard of nonparasitic diseases that constitute the best approach to a general phytopathology.

Whetzel (26) protests against the dominance of the etiological point of view because he contends that it has been responsible for the preoccupation of phytopathologists with fungi and bacteria and for their neglect of anatomical phytopathology. His stricture on etiology is not surprising in view of his definition of etiology as "that phase in the study or discussion of a disease which deals with the chief causal factor, its nature, classification, and relations with the suscept." Suscept, in turn, is defined as "any plant liable to infection by a given pathogene," and pathogene as "any organism capable of causing disease" (25).

Reflection upon these strictures against etiology and its deleterious influences reveals that they do not apply to a *thoroughgoing etiology,* but only to the *pseudo-etiology,* which has dominated phytopathology since the last half of the 19th century. This false etiology arose as a consequence of the quest for single external causes, which dominated the atomistically-oriented natural sciences of the last half of the 19th century and manifested itself in part in the naïve version of the germ theory of disease that characterized the biology and pathology of this period (12). The restricted

definition of the field of etiological pathology, which Morstatt and Whetzel accept, ignores the very important principle that factors within the plant itself are part of the complexes of antecedents out of which pathic events arise, and, in being concerned only with external factors, inevitably deflects attention from the pathic plant itself.

A thoroughgoing etiological pathology considers *all* possible antecedents and recognizes that causal complexes usually include both *internal* and *external* factors. An etiology that recognizes that internal factors are part of these complexes is bound to treat the pathic plant and its processes as the central object of interest and analysis and to give experimental phytopathology a genetical, physiological, and biochemical turn. Precise etiological analysis of any complex pathic event, such as an infectious disease or a pathic form anomaly, compels one to cut the complex event conceptually and experimentally into analytical temporal and spatial isolates and to deal *seriatim* with each isolate and its antecedents. By arranging these isolates in their proper sequence, a more or less complete etiological description is given of the total pathic event. Such analysis, if rigorous, in case of multicellular organisms, invariably leads to consideration of groups of cells or of single cells and of the antecedents of their structures and processes. In other words, a full etiological description is the same as a thoroughgoing narrative of the causal pathogenesis of a pathic event and treats the structures and processes of the affected plant as the center of interest and analysis. Because of its emphasis on cellular processes of the pathic organism, a comprehensive etiology furnishes a unifying basis not only for a general phytopathology but for a comparative pathology, as well (12).

A comprehensive etiology also serves better than anything else to integrate all external and internal factors, including the effects of pathogenic foreign organisms, into a *single etiological scheme* or formula (12). At present most American and English textbooks and special articles treat predisposing factors and the factors of varietal susceptibility as categories coordinate with the category of etiology and not as integral parts of etiology. Consequently, the concepts and data arising out of genetical analysis of susceptibility and out of the experimental study of predisposing factors are not integrated in a single etiological scheme. German phytopathological literature has formally recognized that etiology deals with causal complexes and that a causal complex usually includes conditioning factors (*Bedingungen*) and an incitant (*Erreger*). It is highly desirable that American phytopathologists adopt the term "incitant" or some better equivalent to replace "the cause."

A thoroughgoing etiology, recognizing the rôle of both internal and external factors, will restore constitutional pathology as the keystone of

etiological pathology. With characteristic insight the Greek natural philosophers treated it as such, and from the time of Theophrastus (370–286 B. C.) to publication in 1853 of de Bary's first paper on pathogenic fungi (1), constitution and disposition were key concepts of plant physiology and pathology. Even during the dominance of the germ theory, Sorauer (20) and Ward (24) contended that a genuine etiology must consider the effect of predisposing factors (which they considered to be mainly external ones) upon the disposition of the plant as this expresses itself in normal and abnormal susceptibility to incitants. Latterly, Morstatt (14), in his discussion of physiological pathology, Fischer and Gäumann (5), in their discussion of the prerequisites of infection, and I (12), in a discussion of the rôle of genetics and developmental physiology in etiological pathology, have formally treated constitution and disposition as phytopathological concepts.

Finally, a comprehensive etiology also may serve to integrate experimental phytopathology and the other experimental biological sciences. In fact, the only difference between etiological pathology and each of the other experimental biological sciences is that, while these deal with the relations of the structures and processes characterizing maintenance, development, or reproduction within the range of easy tolerance of the living system in question, their pathological counterparts deal with the relations of those structures and processes that characterize difficulty or failure in maintenance, development, or reproduction (12).

A radically experimental etiology is not content to establish merely that a particular strain of *Puccinia graminis tritici* incites the pathic complex called rust in a particular strain of wheat. It also aims to account for the occurrence of the pathic event under particular past and present external conditions within a particular cell in a particular tissue of a particular age within a particular organ of a particular individual. Furthermore, it aims to explain how the presence of a particular organ of a specific parasite, which is in a particular phase of its life cycle and has a particular genetic constitution and a particular environmental past, incites this particular cellular event.

In order to attain these goals etiological phytopathology must come to grips with the problems of development and must utilize the concepts and techniques not only of the other botanical sciences, but of the nonbotanical sciences as well.

GENERAL ETIOLOGY

Experimental analysis of healthy and pathic events has shown that in addition to the obviously pathogenic external factors every factor that plays a rôle in development and maintenance and reproduction of living systems is a potential pathogenic factor (12). In other words, there is no

special class of pathogenic factors any more than there is a special class of pathic processes. Analysis has also shown that any one of these factors may play a determinative, direct or a factorial, indirect pathogenic rôle. It may act as a releasing, inciting, proximal or as a conditioning factor.

It also has been established experimentally that one factor rarely is so determinative that no other factor plays a rôle. However, such cases do occur. Lethal genes and in some cases sublethal genes are so determinative that other factors within and external to the individual are of practically no consequence. At the other extreme overwhelming external forces often play such a sudden and completely catastrophic rôle that other factors are practically negligible. In most cases, however, the antecedent of the pathic event is a complex of factors, a *causal complex* (13). Many internal and external factors cooperate in the production of an antecedent complex, which manifests itself as a *disposition* on the part of the plant to react pathically to a particular inciting internal or external factor.

The factors of a pathogenic complex may therefore be roughly divided into (a) *Internal, Constitutional Factors,* which manifest themselves in part in the *disposition* of the organization, and (b) *External Factors.*

Internal or Constitutional Pathogenic Factors. The concept "constitution" has had various meanings since its introduction into pathology by the Greek physicians and natural philosophers. Constitution, as used here, means not only the constituent parts, that is, the structures and processes of the organization of a particular individual, but also the individually characteristic manner in which these are spatially and temporally coordinated and integrated to produce the particular organization and behavior or reaction of the individual at any given instant. Sometimes the term constitution is used to designate the sum total of the possible reactions of an organism. The constitution of an organism may be analyzed into (a) its genetic, idio- or genotypic constitution, and (b) its nongenetic, para- or, less correctly, its phenotypic constitution. These do not exist as independent entities. The constitution of an organism as we see and deal with it always is the phenotypic expression or resultant of an interplay of the genetic, the nongenetic internal, and the external factors of the organism.

Genetic Constitution. By genetic constitution is meant not only the comparatively invariant part that an individual received from its parent or parents, that is, its chromosomal and cytoplasmic genes, but also the individually characteristic manner in which these are spatially and temporally coordinated and integrated into a genic system. If the contentions of Correns and Wettstein are correct, that a cytoplasmic genetic system exists in cells, then the genetic constitution of an organism, in addition to its genic constitution, also consists of a cytoplasmic part, the plasmone, and of the relation of the genic and extragenic genetic systems (12).

The direct determinative pathogenic rôle of the genetic constitution of plants is illustrated by the lethal and sublethal effects of certain chromosomal and cytoplasmic genes reported for a considerable number of plants (12). It is also illustrated by the gene effects recognized as sterility and semi-sterility and by many cases of lethal polyploidy.

The indirect or conditioning pathogenic rôle of the genetic constitution is illustrated by innumerable cases of genically conditioned susceptibility of plants to the pathogenic influence of both nonliving and living external incitants, especially of pathogenic foreign organisms.

The phase of etiological pathology that deals with the effects of the genetic constitution of the plant brings experimental pathology into intimate contact with physiological genetics. In analyzing how genes play a direct and/or indirect rôle as pathogenic factors, etiological pathology will find itself compelled to consider the propositions and concepts of physiological genetics (12). It may conceive the gene as a self-synthesizing, self-duplicating catalytic agent, which, in its primary rôle, is the antecedent of synthesis of particular enzymes and/or antienzymes at definite moments in the life history of the organization of which each gene is a part. The enzymes of each gene may be assumed to react with and upon the living substrate and/or such other materials as are present. These enzymes, in turn, in cooperation with the resultants of the action of all other genes, may be considered antecedents of the processes and structures of the cell or acellular entity under analysis. Through the intermediacy of these cells and their products, in interaction with other cells and their products, in case a multicellular organism is involved, the structures and processes of tissues and organs, and even the behavior of the organism as a whole may be considered to be conditioned by the genes. This is essentially the interpretation presented by my colleague, Sewall Wright.

Past-History and Correlative Influences—The nongenetic constitution of an organism is the relatively variable and transitory part of its organization. In the beginning the nongenetic constitution of each individual consists of the nongenetic cytoplasm and the cytoplasmic elaboration products it received from its parent or parents. Later, it consists of new cytoplasms, synthesized in the course of growth and development through interplay of the original nongenetic cytoplasm and of the genetic constitution of the individual as well as of the elaboration products of these cytoplasms. The nongenetic constitution of an organism changes in the course of the life history of the individual as differentiation of cells, tissues, and organs proceeds. It is the repository of all past events in the organism, excepting mutations in the genetic system. It learns by experience, it ages, and eventually it dies and disintegrates unless all or part of it is carried on in the formation of new individuals.

The factor of the past experience or development of the nongenetic cytoplasmic system and its products may be designated as the *past-history* factor (12). Through it, all past experiences and events of the organization tend to play a conditioning rôle in the events of the present moment of an organism. The disposition of an organism is an aspect of its past history. It is assumed that the genetic and nongenetic constitutions of a cell are in constant interplay and that the procession of events whose possibility is determined by a relatively small number of genes is released by correlative influences between genes and changing cytoplasm. It is assumed that this factor is a very important one, making it possible for a limited set of genes to determine all of the potentialities of an organism. Interplay with a changing cytoplasm may enable the same set of unchanging genes to condition new structure and behavior from moment to moment. Examples of the pathogenic influence of the past history factor are seen in those processes of differentiation that lead to death of cells, in the effect called aging, in the metabolic drift of various levels of organization of plants, and in acquired susceptibility to pathogenic agents or factors.

Mutual effects between different parts play a rôle in the constitution of every cell. Similarly, the effects of one cell and its products upon another cell in the same organism constitute a factor that conditions the constitution of the organism. These factors, internal to the organism as a whole, but external to a given call, tissue or organ, are considered the *internal environment* of the organism. They are designated as *correlative influences* or effects (12). They may be positional, mechanical, chemical, electrical, or radiative. They play a leading rôle in conditioning that the progeny of one cell (the zygote) or of a group of cells, all with the *same* genetic constitution differentiate into *diverse* tissues and organs.

The rôle of correlative pathogenic factors is seen in chlorophyll deficiencies due to incompatible nuclear and cytoplasmic constituents; in death of the zygote due to incompatible nucleus and cytoplasm; and in abscission of buds and young fruits following the ill effects of correlative transpiration in which water is withdrawn from the tender tissues by more mature and actively transpiring leaves. It is also seen in the effects of the pathic root system of the peach tree upon its aerial parts in the phony disease of the peach.

Consideration by etiological pathology of the past-history factor and of the factor of correlative influences brings experimental pathology into intimate contact with physiological genetics as well as with developmental physiology and morphology.

Disposition. The constitution of an organism may be evaluated with reference to its capacity or probability of reacting, either adaptively and favorably, or nonadaptively and unfavorably, to a given internal or exter-

nal stimulus, *i.e.*, with reference to its chances of persistence or resistance. The constitution of an individual is such that it is either capable or incapable of being stimulated into pathic reactions by a particular factor or agent. This factor thereby becomes either a pathogenic or a nonpathogenic factor as measured by the reaction of the plant. The plant, organ, tissue, or cell is said to be susceptible or resistant to or immune from pathogenic stimulation by that particular factor or, less correctly, the particular disease incited by that factor. Susceptibility and resistance or immunity, therefore, are relative polar concepts; the greater the one, the less the other.

This relative susceptibility and resistance to pathogenic stimulation are designated as positive and negative disposition (18). The term disposition, which represents a physiological concept and not a material entity, is derived from *disponere,* to be made ready or prepared.[3] When not otherwise qualified, the term disposition usually means *positive disposition* or susceptibility. In evaluating susceptibility and resistance, the usual behavior of the organization in question (species, individual, organ, tissue, or cell), with reference to its usual internal or external factors, is often taken as the norm. Disposition, consequently, is often defined as an exaggerated or abnormal reaction to stimuli.

In so far as the disposition of an organism is genetically determined or conditioned it may be said to be a hereditary, geno- or idiotypic disposition. This manifests itself especially in the differential susceptibility and resistance of different genotypes and in varietal, species, generic, and familial dispositions. It is exploited practically in selection and breeding for "disease resistance." The expression of disposition which is conditioned by nongenetic factors may be designated as nonhereditary, pheno- or paratypic disposition. It manifests itself in the individual dispositions elicited by particular environmental factors and in dispositions correlated with age, sex, organs, and external factors. An absolutely sharp line cannot be drawn between genotypic and phenotypic disposition because the actual or phenotypic disposition of an organization is always a resultant of an interplay of its genetic and of its nongenetic internal and external factors. Sometimes the genetic constitution plays such a determinative rôle that a disposition is essentially genotypic. Disposition represents the particular manner in which an organism reacts to any given stimulus at any given moment. It is the result of an integration and summation of the effects of all factors that have influenced the organism previous to that moment. Essentially, it is the past-history effect.

[3] The term diathesis (διάθεσις), a preparation, arrangement, has essentially the same meaning as disposition. Sometimes it is restricted to those dispositions that are primarily due to the chemico-physical organization of the tissues.

Sorauer (20) designated as *predisposition* the expression of unusual susceptibility by individuals of the same variety in response to environmental factors. Morstatt (13) designates individual phenotypic susceptibility as predisposition and reserves the term disposition for genotypically determined susceptibility. Fischer and Gäumann (5) use the term susceptibility (*Empfänglichkeit*) for both genotypically and environmentally conditioned individual susceptibilities, but restrict the term disposition to the latter.

External Pathogenic Factors. The class of external pathogenic factors or agents comprises all pathogenic factors that influence a living organization from without. They may be effective either singly or in combination and directly or indirectly. Every type of environmental factor is potentially pathogenic for some organization at some place, at some time. Under certain conditions of intensity, duration, and even of occurrence or nonoccurrence, a given factor may be antecedent to a healthy reaction; under others, to a pathic reaction.

Nonliving External Agents. There is no need to discuss here the various agents that comprise the categories of physical and chemical pathogenic factors. Plant pathology and, to a greater extent, certain phases of physiology and ecology, have devoted themselves to study of this phase of etiological phytopathology. By wholehearted consideration of the rôle of these factors as conditioning agents of susceptibility to pathogenic infection and as direct determinative pathogenic factors, experimental pathology comes into intimate contact with physiology and ecology.

Living External Agents. If one of the significant external factors or agents is another organism, as in predatism, pathogenic epiphytism, pathogenic parasitism, and pathogenic infection, the pathogenic activity of the foreign organism must be analyzed into its determinative and/or its conditioning factors. The attacking organism itself is an event like an attacked one, and the interplay of the stimuli and reactions of both of these organisms constitutes the most complex type of event with which the pathologist has to deal.

The relative capacity of a predator to attack, *i.e.,* its predativity, and of a foreign organism to infect or to infest, *i.e.,* its infectivity or its infestivity, may be included in the general concept "*aggressivity.*" The relative capacity to incite a pathic event may be designated as *pathogenicity* or specifically in cases of microorganisms as *virulence.* Aggressivity and pathogenicity may be considered an aspect of the disposition of an organism and are measured by the extent to which it exploits the susceptibility or overcomes the resistance of the organization with which it becomes associated. Susceptibility and resistance, on the one hand, and aggressivity and pathogenicity, on the other hand, are reciprocal or polar values or terms.

Aggressivity and pathogenicity at any given time and place are determined and/or conditioned for a given foreign organism or its parts by (a) its internal factors, *i.e.*, factors of its own constitution and (b) its external factors, of which the constitution or disposition of the attacked plant, by virtue of its susceptibility or resistance, is the most important one. At times, factors external to both organisms affect the foreign organism directly. The internal factors for the foreign organism are its genetic constitution, its past-history factor, and the factor of correlative influences.

If the genetic constitution is the sole or determinative factor in aggressivity and pathogenicity these may be designated as hereditary, genotypic, or idiotypic aggressivity and pathogenicity. The actual phenotypic aggressivity and pathogenicity manifested at a given time and place, however, are resultants of an interplay of internal genetic and nongenetic factors and of external factors. Fischer and Gäumann (5) distinguished between (a) innate, constitutional differences in aggressivity and (b) changes in aggressivity, which they call disposition. As pointed out previously, it seems preferable to use this term to represent the phenotypic expression of the resultant interplay of internal and external factors. The aggressivity and pathogenicity of an organism may be correlated with age, sex, taxonomic relationship, nuclear phase, developmental phase, as well as with definite organs, tissues, or cells of an individual.

The phase of etiological pathology that deals with pathogenic parasites brings experimental pathology into intimate contact with parasitology, especially with its bacteriological, mycological, nemathelminthological and entomological aspects. In so far as it deals with the internal factors that condition the aggressivity and/or pathogenicity of predators and parasites it brings experimental pathology into intimate contact with the genetics and developmental physiology of these organisms. Since the time of de Bary, etiological phytopathology has been predominantly parasitological, and parasitism and infection have been its leading concepts.

SUMMARY

For the sake of convenience the factors or agents that may condition and/or incite a pathic event within an acellular[4] or unicellular organization or within any given part of a particular tissue or cell of a multicellular organism may be summarized in the following tabular form:

I. Internal factors (factors within the organism)
  A. Genetic constitution
    1. Chromosomal genes
    2. Cytoplasmic genes
    3. Nongenic genetic factors (plasmone)?

[4] By acellular is meant a living organization not involving a nucleus and cytoplasm.

B. Past history
   1. Original nongenetic constitution
   2. Acquired nongenetic constitution
C. Correlative influences
   1. Between parts of the same cell or of an acellular organism
   2. Between different cells or tissues and/or their products

II. External factors (factors outside the organism)
A. Physical agents
   1. Light and other rays
   2. Heat
   3. Cold
   4. Electricity
   5. Radiations
   6. Mechanical-traumatic agents
B. Chemical agents
   1. Toxic substances
   2. Enzymes
   3. Hormones (growth-promoting and inhibiting substances)
   4. Nutritive and metabolic substances
C. Organized living agents
   1. Organisms effective without contact
   2. Organisms effective by temporary contact (predators)
   3. Organisms effective as epiphytes and epizoa
   4. Organisms effective as parasites (plant and animal)

The aggressivity and pathogenicity of each pathogenic foreign organism must be analyzed so far as possible into their determinative and/or conditioning factors, which are of the same kind as those listed in categories I and II. To conserve space these are not repeated for each subcategory of category II C. Schemata that diagrammatically represent the possible influences of these factors as antecedents of a given pathic event in a given place at a given moment have been presented in an earlier paper (12).

II. INFECTION AND PARASITISM

a. *Infection*

It is obvious that adoption of a thoroughgoing etiology removes infection and parasitism from the center of etiological pathology. They nevertheless are such important aspects of etiology that they deserve special consideration.

The term *infection* is derived from the Latin verb *inficere,* which means to put in, taint, pollute, defile, contaminate. In present-day biology the term has come to mean: the act of communicating a foreign organism, specifically, a microorganism; the foreign organism which is communicated; the state of harboring a foreign organism; the event of interaction between the foreign and the contaminated organisms; the pathic reaction of the contaminated organism to the presence of the foreign organism; the act of

communicating disease; a disease which is communicable. In this discussion the term infection is used to designate the *state of being contaminated* by a minute foreign organism. Typically, this contaminating organism is microscopic; at least when it lodges upon and/or enters and permeates the affected organism. At one extreme, as in contamination by filterable viruses, infection therefore grades imperceptibly into intoxication by poisons; at the other, as in the parasitism of mistletoe, it grades imperceptibly into infestation, that is, contamination by a large foreign organism.

The term *contagion* has essentially the same meanings as infection. It is derived from the Latin verb *contangere,* which means to touch. Sometimes the term *contagium* is used to designate that which is communicated, while contagion is used in contradistinction to express the act of communicating. Contagion is also defined as infection by contact.[5]

b. *Parasitism*

Many infections involve the event of parasitism. The concept parasitism has been variously and contradictorily defined. The definition here used is an adaptation of one given by Thompson (21). If an organism of one kind lives on or within a living individual of the same or of another kind, the host, in a one-sided nutritive relationship, which is not beneficial and is often harmful to the host, the individual is said to be a parasite. The concept parasitism, therefore, is formulated to apply at the individual or species level of organization of biological entities. It is, therefore, essentially an ecological concept. The organism on or within which the parasite is living and from which it derives its food is said to be parasitized. The relation of parasitism relieves the parasite from most of the activity and struggle usually associated with procuring food and thus tends to induce some degree of simplification or degeneracy. *One-sided food relationship* between two associated individuals, therefore, is the essence of parasitism. This was the original implication of the term. In Greek society a person who was assigned by social custom and law to eat at the table of another was designated as a parasite (παρασιτος). The biological concept implies more than a space relationship, but it does not necessarily imply a biologically significant harmful effect, even though it precludes a mutually beneficial relation.

The first detailed discussion of parasitism of plants is found in the "Causes of Plants" by Theophrastus (27). In his analysis of the relation of mistletoe to trees he recognizes the mistletoe as a separate, independently propagating plant whose seeds are disseminated by birds. Theophrastus

[5] The history of these concepts will be published as a separate paper, "Pathic Events in Plants and the History of Infection."

recognized that the mistletoe lives at the expense of the infected tree and that this association often leads to harmful effects for the tree. In this first phytopathological classic on parasitism Theophrastus does not transfer the term parasite from the social to the botanical vocabulary. It is significant that he does not discuss the mistletoe in the chapters devoted to anomalies, injuries, diseases, and death of plants, but in the course of his discourse on the nutrition of trees. He compared its nutritive relation to its host with that of a scion to its stock. He did not, of course, know that the green leaves synthesize part of the food of the scion. Theophrastus also recognized that dodder, orobanche, and fleshy fungi live attached to and at the expense of other plants, often with fatally pathogenic effects.

Tubeuf (23), in his excellent monograph on the mistletoe, lists examples of superstitious beliefs and folk and medical lore of various peoples of Europe about this interesting plant. These show clearly that the anomalous mode of life of the plant rendered it an object of early and special interest to prescientific man. The mistletoe is listed in the earliest herbals of Europe. Matthioli (1586) considers it a self-propagating plant. Later (1679), Malphigi studied the anatomy of the mistletoe and considered it a parasite that incites galls. He does not, however, designate it by the term parasite. The mistletoe apparently was not called parasite in the scientific literature (although early German literature designates it Schmarotzer) until the 17th century.

Sarauw (19) states that Micheli (1729) introduced the term parasite into scientific literature in connection with his discussion of the Balanophoraceae. Micheli, in fact, first used the term parasite in 1720. The first record I have been able to find of its use in botanical literature is by Ray (16) in his Historia Plantarum of 1688. The close of the general discussion of the first volume (1670) of this significant treatise is devoted to a thoroughgoing naturalistic discussion of Diseases of Plants. In discussing the mistletoe, Ray says "Viscus est planta parasitica."[6] Ray, quoted by Duhamel (3), also reports that the London apothecary Doody succeeded in growing the mistletoe from seed and thus demonstrated experimentally that it is an independent self-propagating plant. Tournefort (22), in his Memoir on Diseases of Plants of 1705, in the course of a discussion of the external incitants of disease, including fungi, designates a considerable assemblage of plants, including dodder and mistletoe as parasitic plants.

The first record of unequivocal application of the term parasite to a definite fungus that I have been able to find occurs in a memoir by Duhamel (2) in 1728 on the decay of Safron. In this phytopathological

[6] A reference by Guettard (8) to Morison's Plantarum Historia Universalis Oxoniensis may indicate that Morison may have applied the term parasite to certain plants in 1672 or 1680.

classic the term parasite is applied to a microfungus, which Duhamel considered a truffle-like plant, now designated as *Rhizoctonia crocorum.* Duhamel proved experimentally that this fungus is able to live in the soil; that it may grow into and live at the expense of the safron bulb; that it may incite decay of the scales with attendant death of the entire plant. He showed that it could parasitize other Liliaceae and Amaryllidaceae. He suggests isolation, rotation, and sanitation as control measures. This paper, which is a landmark in parasitological mycology and plant pathology brought Duhamel election to the Royal Academy. The fact that Tournefort (1705) and Duhamel (1728) use the verb parasitize, the adjective parasitic, the noun parasite, without the type of discussion usually attending introduction of new concepts and terms, may indicate that these terms had been used previously in French scientific literature. Tournefort merely says, after listing dodder, mistletoe, and other plants, "With good reason these are called parasitic plants." The term parasite was part of early literary French and English. Rabelais (1495–1533), Shakespeare (1564–1616), and Ben Jonson (1573–1637) applied it to certain characters of their plays.

Guettard (8), in a memoir on the adherence of dodder to other plants (1744) and (9), in a memoir on parasitic plants (1746), recognized levels of parasitism ranging from slight to full food dependence of the parasite on the host. In 1762 Duhamel (4) devoted Book V, Chap. I, Art. V, of "La Physique des Arbres" to a discussion of "Des plantes parasites." By 1767 Fontana (6) recognized rusts of wheat as parasitic, self-propagating pathogenic fungi.

It is contended in some quarters that *Rhizopus nigricans,* which often grows on or within the storage root of the sweet potato, and derives its food from this organ, or *Fomes pinicola,* which often grows within and derives its food from the dead heart-wood of living trees, should not be regarded as parasites because they invade only through wounds and derive their food only from dead cells. This interpretation of the concept of parasitism has led to much confusion. It is based in part upon a failure to recognize that a concept formulated to apply to an organism as an individual or whole is not necessarily applicable to its tissue or cellular level of organization. The essence of the concept of parasitism, in so far as nutrition is concerned, is dependence of *one individual* upon another living individual for food, irrespective of whether the materials used by the dependent individual or parasite are actually derived from the living or dead parts of the host. Consequently, a heterotrophic organism such as *Rhizopus nigricans* or *Fomes pinicola* may be considered a parasite in that it obtains its food from

a living individual[7] even though it actually uses only nonliving constituents of the host.

It is convenient, therefore, to recognize different food habits in parasites. One group of parasites derives its food from the living tissue of the host, *i.e.,* from living cells and/or their immediate products, such as the cell walls. The other group, while living on and at the expense of their hosts, derive their food from dead tissues and cells and their products. The first group may be said to be living biotrophically (βιος, bios = living; τροφή trophe = food), or as biotrophic parasites. The second group of parasites may be said to be living necrotrophically (νεκρός, necros = dead) or as necrotrophic parasites. If one prefers, with Münch (15), to distinguish between those necrotrophic parasites that kill tissues before they use them as food and those that utilize as food the tissues killed by agents other than themselves, one may say that the former carry on nutrition thymatrophically, or as thymatrophic parasites (θῦμα, thyma = victim), and the latter saprotrophically or as saprotrophic parasites (σαπρός, sapros = rotten). The latter term is applicable only because in biology sapros has come to mean more than rotten, being used in the sense of nonliving. Thus an organism growing on a dead body or on an artificial medium is currently designated as a saprophyte. The first group includes all of those pathogenic parasites that kill and destroy tissues by means of toxic by-products and enzymes prior to their invasion and utilization as food. An organism living as a parasite may, therefore, obtain its nutrients either biotrophically or necrotrophically. These terms recognize the differences that exist between *Puccinia graminis,* which lives on the living tissues of wheat, and *Fomes pinicola,* which lives on the dead heart wood of a standing, living tree. In the latter case the presence of the parasite has potential pathogenic implications for the tree as a living whole, even though, considered at the cellular level of the host, the parasite is living only at the expense of the dead tissues.

The concept infection, therefore, is a broader one than parasitism. All cases of parasitism are infections or infestations but not all infections involve parasitism. Thus the epiphytism of microorganisms is a phase of infection but not of parasitism. Furthermore, not all parasites are pathogenic; hence, one must distinguish between pathogenic and nonpathogenic parasites. The flagellate *Herpetomonas* spp., which live in the latex of *Euphorbia* spp. are nonpathogenic parasites.

If an infection is part of the antecedent complex of a pathic event it is a *pathogenic infection* and the resultant is designated as an *infectious disease.*

[7] Because of the low level of organization of plants certain plant organs such as a sweet-potato storage root and other parts of plants capable of continued existence when severed from the plant as a whole may be considered individuals.

This use of the term infectious disease differs from the usual meaning of the term that emphasizes communicability or capability to communicate. In fact, common usage with its reflection of the ancient anthropomorphic notion that diseases are discrete entities is incorrect in the light of biological conceptions about the nature of pathic events. In reality, as pointed out by Henle in 1840 (10), only the infective pathogenic agent, *i.e.*, the incitant, is communicable; the pathic processes, the essence of the pathic event, are not transmissible.[8] If the infective agent is transmissible by contact alone the resultant pathic event is often designated as a contagious disease in contradistinction to infectious diseases, in which the infective principle may be communicated by carriers such as other organisms, soil, water, and air. In common usage, however, the terms contagious and infectious diseases frequently have essentially the same meaning.

Obviously not all infections are pathogenic. Many individuals of the heath family harbor various mycorrhizal fungi (17), *i.e.*, they are infected. However, some of these infected plants, as individuals, do not display any pathic reactions: in fact, the association is supposed to be of actual benefit to them. Nevertheless, particular infected cells may be pathic in that they degenerate and die prematurely because of the infection. Here again, it must be borne in mind that an infection may be nonpathogenic when evaluated with reference to an organism as a whole but pathogenic when evaluated with reference to particular organs, tissues, or cells of this individual. Thus the legume plant as a whole may be benefited by an infection of nodule-inciting bacteria, whereas particular cells in the root are necrotized or incited to pathic development and reproduction. These and the wound responses of many other cells not invaded, which often differentiate into new kinds of tissues, give rise to the gall. All gradations of harmful and beneficial effects to the plant as a whole are exhibited by nodule-bearing legumes. It appears that the bacteria are not able to readily invade the roots or establish themselves unless a soil-nitrogen deficiency so seriously disturbs the carbohydrate-nitrogen metabolism of the plant that it may be considered pathic. A benefited infected plant may, therefore, be considered as a plant that was pathic that became locally infected as a consequence but that, by virtue of the secondary effects of the local infection, overcame the original pathic state. Additional data are found in a monograph by Fred and associates (7).

In infectious diseases, as well as in diseases incited by the larger foreign organisms, the incitant releases chains of reaction or initiates processes in the infected or infested plant that in turn set up stimulus-reaction chains in the infective or infesting agent. These reactions manifest themselves in

[8] It is possible that some chlorophyll deficiencies involve transmission of a pathic cytoplasm from mother to progeny through the megagamete.

part in the susceptibility and resistance of the infected plant and in the infectivity or invasiveness and virulence of the infective agent. The resultant is the most complex type of pathic event found in living systems. It consists of process complexes that manifest themselves in regressive, necrotic, repressive, and/or progressive pathic changes in both the infecting and infected organism. These process complexes consist of processes that differ in no way from those that characterize pathic events whose etiological complex does not include an infective agent or from those that characterize healthy events. They differ from those of healthy events largely in that the duration, intensity, and combination of the constituent processes are characterized by lack of regulation and integration.

III. ETIOLOGICAL CLASSIFICATION OF PATHIC EVENTS

Beginning with the taxonomic phase, which dominated biology during the 18th century, attempts have been made to classify pathic events as though they were discrete entities. Pathic events do not exist apart from individual living entities and hence can be classified only if they are abstracted into concepts that generalize the particular events occurring within individual organisms. Such abstraction, however, is necessary if the maze of our sense experiences about pathic events is to be coordinated into a convenient system and if relations are to be formulated and a scientific presentation made possible.

Etiology concerns itself in part with the classification of antecedents of pathic events, but it is not concerned with the classification of pathic events themselves. Etiology, however, has furnished several bases for classifying pathic events that have proved more convenient than systems based on other criteria. Consequently etiological classifications of pathic events have become and probably will remain dominant in phytopathology.

In consonance with the classification of pathogenic factors previously given, pathic events may be grouped as follows:

I. Pathic events incited or determined by internal factors
   - A. By genetic factors
   - B. By nongenetic factors.

II. Pathic events incited or determined by external factors
   - A. By physical agents
   - B. By chemical agents
   - C. By organized agents
     1. By organisms effective at a distance
     2. By organisms effective by temporary contact (predators)
     3. By organisms effective as epiphytes and epizoa
     4. By organisms effective as parasites.

In this classification the phrase "pathic event incited or determined by" is to be read to mean a *pathic event in whose etiological complex* a particular factor plays the inciting or determinative rôle.

Pathic events are grouped most frequently as nonparasitic and parasitic pathic events (diseases). This system or variants of it have long been in vogue and are widely used today. In the classification just given, all categories, excepting II 4, represent non-parasitic pathic events. If "parasitic disease" is defined to mean any pathic event whose etiological complex includes a parasite, *i.e.*, as a pathic event that is incited or determined by a parasite, and "nonparasitic disease" is defined to mean any pathic event whose etiological complex does not include a parasite, i.e., as a pathic event that is not incited or determined by a parasite, these categories are etiologically sound and their use does not necessarily stifle the development of general cellular phytopathology nor split the field of phytopathology into applied botany and applied zoology. This results only if the *content or subject-matter* of phytopathology is delimited on an etiological basis and is avoided if the essence of the concept "pathic event" is taken to be a *harmful occurrence* within an organism rather than an occurrence brought about by a *particular kind* of factor or agent, or by a factor that is not a *usual part* of the environment of the affected organism (12).

IV. ETIOLOGICAL CLASSIFICATION OF CONTROL MEASURES

An etiological classification of pathic events that recognizes that factors both within and without the plant may play inciting and or conditioning pathogenic rôles gives a basis for classification of control measures that is of use in general plant pathology. Control measures, on this basis, may be placed into one category that comprises those measures that are directed primarily or solely at the potentially or actually pathic plant as a pathogenic factor and into a second category that comprises those directed primarily or solely at factors outside the potentially or actually pathic plant, as pathogenic factors.

UNIVERSITY OF CHICAGO,
CHICAGO, ILL.

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CERTAIN SCLEROTIUM DISEASES OF GRAINS AND GRASSES[1]

RUTH REMSBERG AND C. W. HUNGERFORD

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INTRODUCTION

A fungous disease, which appeared in the wheat fields when snow was melting, was first reported in Idaho (4) in the spring of 1922 in Fremont and Teton Counties. This disease has occurred in this region with more or less severity each year since then. It was of considerable importance in 1931 and 1932 on the University of Idaho Substation Farm at Sandpoint, Idaho, and was reported in 1931 from near Hill City, Idaho, and in 1932 from near Cascade, in Long Valley, Idaho. This same disease has been reported on wheat from Washington[2] and Montana[3,4] and has been found on lawn grass in Ithaca, New York,[5] and at Moscow, Idaho. Injury from apparently the same disease is noticed almost every year in northern parts of Japan (1, 5). It has been found in northern Germany (3, 6, 7) and in the Scandinavian countries (2).

The disease is most commonly found at high altitudes where winter wheat is grown and in localities where the snow disappears late in the spring.

DESCRIPTION OF DISEASE

The disease was first attributed to snow injury but later a fungus was found always associated with it. The plants seem to be infected under the snow, since portions of the leaves appear scalded and discolored in spots when the covering of snow is taken away. After the snow melts, these leaves die and become greyish white, on drying, and finally become matted together on the ground. When a field is heavily damaged, entire areas are covered with greyish white dead plants. When the damage is slight, green leaves may be seen here and there. As the weather becomes warmer, the activity of the fungus ceases, and the partially infected plants grow vigorously, and often the effects of the disease are gradually overcome. Because of its gen-

[1] Published with the approval of the Director as Research Paper No. 101 of the Idaho Agricultural Experiment Station.

[2] Heald, F. D. *Sclerotium rhizodes* Avers. U. S. Dept. Agr. Bur. Pl. Indus. Pl. Dis. Rptr. Suppl. 35: 270. 1924.

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[4] Young, P. A., and H. E. Morris. Sclerotium root rot (*Typhula graminum* Karst.). U. S. Dept. Agr. Bur. Pl. Indus. Pl. Dis. Rptr. Suppl. 69: 127. 1929.

[5] Dr. H. H. Whetzel of Cornell University kindly sent the writers a specimen of lawn grass on which were found a few sclerotia. A culture was secured from these sclerotia.

eral appearance in the field, it has been called "snow-mold" and "snow-rot" by Japanese workers (1, 14, 15). Due to the scalded appearance of the plants as the snow melts, the writers suggest the name "snow scald."

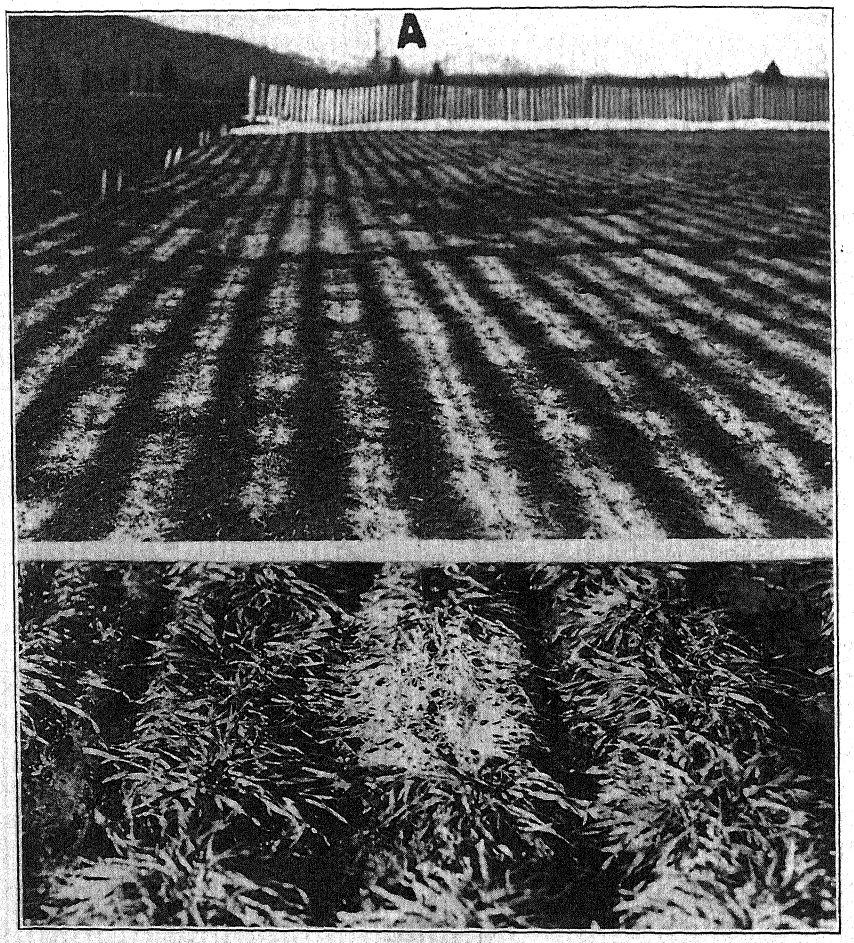


FIG. 1. A. Winter cereal plots on the University of Idaho Substation Farm at Sandpoint, Ida. These plots were almost entirely destroyed by the snow-scald fungus. B. Healthy and diseased Mosida winter wheat plants soon after the snow had melted. Note that only a portion of one plant was diseased. Photographs by J. H. Christ.

In the first stages of the disease, the infected parts are covered with a white growth and, later, brown sclerotia appear in abundance throughout the infected tissue. After the snow disappears, layers of white, mouldy growth are found on the soil and infected plants. (Fig. 1, A.) Soon the infected leaves lose their green color, become dry, and lie matted on the

ground. Some of the inner leaves may still be green and the plant only partly killed. The leaves, stems, crowns, and roots of the plants may show signs of infection. The roots of the infected plants are often found rotted, and mycelia appear on them near the surface of the ground. Often the leaves, stems, and other infected parts are covered with sclerotia that

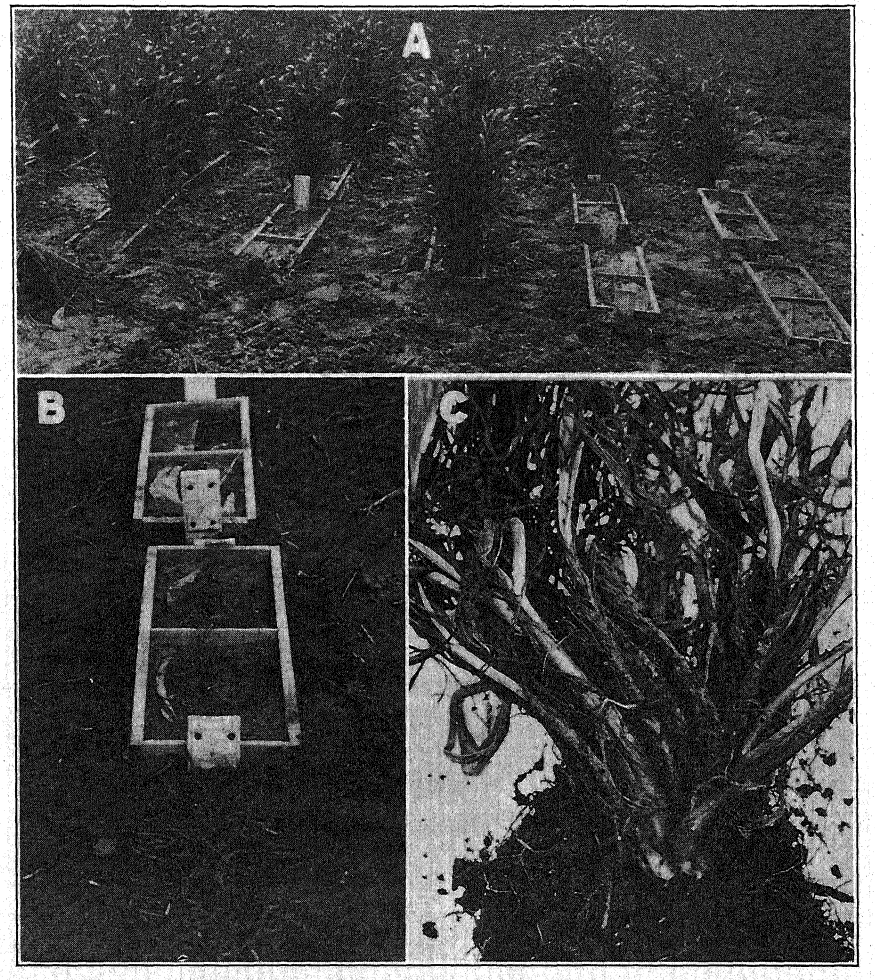


FIG. 2. A. and B. Wooden frames used to test the pathogenicity of various cultures of fungi. Cultures containing sclerotia were added to the soil inside the wooden frames in the spring. The frames were then covered with cloth during the summer. Wheat was planted Sept. 25 in the frames and in the noninoculated soil adjacent as a check. Note that nearly all plants were killed in the frames. C. Infected winter wheat plant taken from the field early in April. Note the numerous sclerotia embedded in the tissues.

range in size from an ordinary pinhead to several times as large. (Fig. 2, C.) Early in the season the sclerotia are yellow or light brown but turn dark later. When mature they are ovoid to spherical, dark brown, and of irregular, rough contour. They are often massed together. They are fastened so securely to the infected plant that it is almost impossible to remove them without tearing the tissue. These sclerotia may also occur on the roots an inch or two below the crown and a great many of them are often found embedded within the leaf sheaths. They are very numerous on the leaves of the dead plants or on plants that have been partially killed. On the partly killed plants light brown, spotted areas appear along the inner leaves, while the outer areas die. The fact that portions of the plant may be attacked or killed by the fungus while the rest of the plant is apparently healthy, has been considered good field evidence of the pathogenicity of the fungus. (Fig. 1, B.) During the summer, these plants, dwarfed and weakened by the disease, are found scattered over or along the edges of the infected areas. Only a few short heads develop upon such plants and the kernels of wheat produced are badly shrivelled. The dead areas in the field correspond to places where the snow disappeared late in the spring. Since the plants become infected during the winter, it is thought that the sclerotia, which fall to the surface of the ground, or remain on the stubble in the field, may be the source of infection the following year.

ECONOMIC IMPORTANCE

The disease ranges in severity from a slight trace to a total loss. In high, dry fields the damage is only slight, while in low, moist places or where the snow remains on the field late in the spring, the damage is very severe. In the fields in Teton and Fremont Counties, in 1923, the damage ranged from a trace to 50 per cent. At the Sandpoint Substation in the experimental plots of wheat and barley, in the spring of 1931, the damage ranged from 5 to 85 per cent. In some cases the yield was reduced 75 per cent. When the disease is very severe, the fields often have to be reseeded to spring wheat. In 1922, 1926 1929, and 1932 snow scald caused varying amounts of damage in certain areas of Teton and Fremont Counties. In each of these years from several hundred to several thousand acres of winter wheat were killed and had to be reseeded in the spring.

EXPERIMENTAL DATA

The causal organism may be easily isolated from either the sclerotia, which are formed so abundantly in the infected parts of the plant, or from the infected plant tissue, themselves. The fungus grows readily upon various media, and sclerotia form abundantly from 1 to 4 weeks later.

Specimens of diseased plants or cultures of organisms from grains and grasses have been obtained from 10 different sources.

1. Culture No. 226 came from material sent to the experiment station from Drummond, Idaho, (altitude approx. 6,000 ft.) in 1926.
2. Culture No. 227 was isolated from diseased wheat plants from Felt, Idaho, (altitude approx. 6,000 ft.) in 1930.
3. Culture No. 278 was obtained in 1931 from diseased barley plants from the Sandpoint-Substation variety-test plots (altitude approx. 2,127 ft.).
4. Culture No. 279 was obtained from Hill City, Idaho, (altitude approx. 5,200 ft.) in 1931 from a portion of a wheat field where snow had remained rather late in the spring.
5. Cultures No. 280 and No. 281 were isolated from 2 diseased specimens of wheat from fields at Felt, Idaho, in 1931.
6. Culture No. 282 was obtained from diseased grass from Ithaca, New York, in 1931.
7. Cultures Nos. 283, 284, 285, and 286 were obtained from Heizi Tasugi, Tokyo, Japan, in 1931. These were isolated in 1926 and 1928 from barley, wheat, timothy, and foxtail grass (*Alopecurus fulvus* L.).
8. Culture No. 287 was obtained from grass from a lawn in Moscow, Idaho, (elevation 2,600 ft.) in 1932.
9. Culture No. 288 was obtained from orchard grass (*Dactylis glomerata* L.) on the golf course in Moscow, Idaho, in 1932.
10. Culture No. 289 was isolated from diseased barley in the variety test plots at the Sandpoint Substation in 1932.

TABLE 1.—*Diameter of vegetative mycelia and size of sclerotia of the organisms in each of the 4 groups of fungi*

Group	Culture	Diameter of vegetative hyphae	Size of sclerotia
	No.	μ	*Diam. in mm.*
I	226	2.44 – 3.33	0.1 – 0.25
	277	4.22 – 4.88	0.9 – 2.0
II	279	2.89 – 4.44	0.9 – 1.2
	281	3.55 – 4.44	1.4 – 2.0
	278	2.88 – 4.44	1.0 × 1.5 – 1.9 × 2.0
	282	3.21 – 4.66	1.8 × 2.0 – 2.0 × 4.0
	283	3.55 – 4.44	2.0 × 2.3 – 2.5 × 2.5
III	284	3.77 – 4.66	1.4 × 2.0 – 2.0 × 2.2
	285	2.89 – 3.77	1.6 × 1.6 – 2.0 × 2.3
	286	2.44 – 4.22	2.0 × 3.0 – 2.5 × 2.9
	287	3.33 – 4.44	1.1 × 1.5 – 1.7 × 2.0
	288	3.55 – 4.44	1.5 × 1.9 – 1.5 × 2.0
	289	3.21 – 4.44	1.2 × 2.0 – 2.0 × 2.2
IV	280	4.44 – 6.66	4.0 × 5.0 – 6.0 × 10

These 14 sclerotia-forming fungi have been studied carefully. Observations on their growth characteristics and reactions to environmental conditions have resulted in classifying these various isolations into 4 different groups, those in each group having approximately the same characteristics.

MORPHOLOGY

When a sclerotium germinates, it sends out white, branched, septate mycelia. These mycelia at first adhere closely to the surface of the medium,

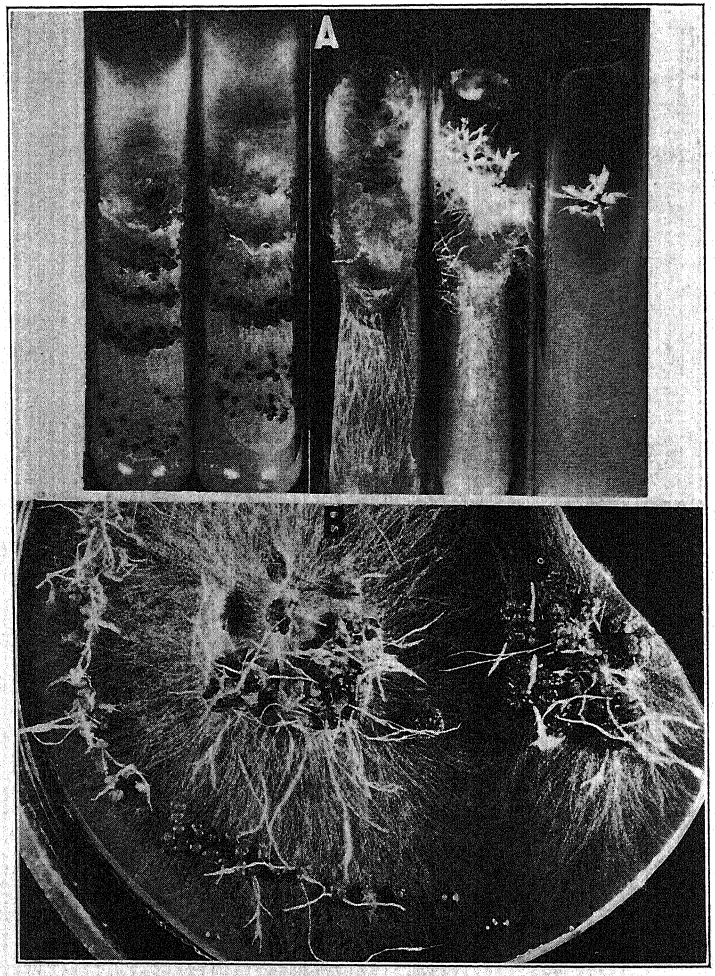


FIG 3. A. Cultures showing characteristic progressive development of sclerotia on potato-dextrose agar slants. B. 138-day-old culture No. 278 growing on potato-dextrose agar. This photograph illustrates the typical fluffy mycelia and the thread-like growths composed of compact masses of mycelia growing from the sclerotia.

but later become aerial and fluffy. The hyphal septae may be either at right angles or oblique to the long axis of the hypha. In cultures belonging to groups II and III the septae are accompanied very frequently by clamp connections. The long hyphae branch and rebranch many times. Growth is very slow in group IV as compared with the other groups. The content is more or less granular and the cells often appear vacuolated. Sclerotia are formed by a dense cluster of hyphae whose cells become somewhat enlarged. The young sclerotia seem to originate from a single hypha or from two to several knotted together. Sclerotia may form in a central mass in culture or in concentric rings or profusely scattered over the surface of the media. (Fig. 3, A.) They are often accompanied by honey-colored drops of watery secretion. In group I, the sclerotia appear both rapidly and profusely and are separate and distinct from each other. They have no rind in cross-section, are light brown to black when mature. They are spherical to oval in shape and smooth. The sclerotia in group II do not appear so rapidly as those in group I, and may appear agglomerated. They have no rind in cross-section, are spherical to irregular in shape, and either smooth or rough. They are light brown when young and dark brown when mature. Sclerotia in group III appear with about the same rapidity as in group II but often coalesce into large spongy masses. They are pinkish orange and gelatinous when young and reddish brown with a tough rind when mature. They are rounded to irregular in shape and superficially rough. The sclerotia in group IV are very slow to appear in culture and are often massed together. They are milky white when young, black when mature, and have a heavy black rind that surrounds a white medullary area in cross-section. They are large and irregular and are often cup-shape on one side.

Very often sclerotia will develop long, slender, thread-like rhizomorphs composed of compact strands of hyphae. (Fig. 3.) In cultures belonging to group II, these growths resemble brown, horn-like, simple or branched projections 5–15 mm. long. In cultures belonging to group III, they resemble long simple or branched white threads 5–50 mm. long. These same rhizomorphs develop in the field during summer and early autumn from sclerotia in the soil.

PHYSIOLOGY

Various media have been used in culturing the organisms, but potato-dextrose agar has thus far given the best vegetative growth and sclerotial development in almost every case.

The sugar content of potato dextrose was changed by adding Karo syrup in varying amounts. The cultures were grown also on media containing synthetic nutrient solutions (9) in an attempt to induce sporulation.

Culturing on acid media also was tried in an attempt to produce the perfect stage (10).

The organisms react very definitely to variations in temperature. Those in group I attained optimum growth at 10° C., those in group II at 5° C., those in group III at 0°–5° C., and that in group IV at 5° C. In all instances growth was better in the dark than when any light reached the cultures. Growth was greatly diminished at temperatures from 10° C. to 20° C., while the organisms ceased growing entirely at temperatures higher than 25° C. Because of this maximum growth of the fugus at low temperatures in culture and the occurrence of the disease associated with snow and cold weather in the field, it would appear, then, that the maximum development of the fungus is definitely favored by cold weather.[6]

Sunlight, also, seems to have a pronounced influence on the growth of the fungus. Mycelial growth is retarded and sclerotia fail to develop in cultures that have been exposed to direct sunlight. Diffused light retards growth but the effect is less pronounced than is that of direct sunlight. Tasugi (14), however, who has done extensive work with the organism in Japan, has shown that sunshine plays an important rôle in the production of basidiospores. He reports that sclerotia, exposed to sunshine while in the soil, will produce hymenophores bearing basidiospores.

In attempting to obtain the perfect stage of the fungus, cultures were exposed to both direct sunlight and to ultra-violet light. Exposures to direct sunlight were given between the hours of 11:00 A. M. and 3:00 P. M. and were of varying duration. However, no tendency toward sporulation was observed.

Stevens (11, 12, 13) and Ramsey (8) have been able to produce the perfect stages of various fungi by exposure to ultra-violet light. Cultures of sclerotia growing on nutrient media and also soil, containing sclerotia from the field, were exposed to ultra-violet light for different lengths of time at several distances from the source of light. Exposure both to sunlight and ultra-violet light were made through Cellophane, which was sealed over the dishes in order to keep the cultures sterile during the time of exposure. Cellophane was used because ultra-violet rays will pass through it, while they are screened out by ordinary glass. Some of the cultures of Nos. 278 and 285, when exposed to ultra-violet light, produced a very few growths resembling sporophores and originating from the sclerotia. A few tiny spore-like bodies were seen associated with these growths. Although the experiment has been repeated, no sporophores developed. This is the only indication of the perfect stage yet found in any of the cultures. This evi-

[6] Observations indicate that damage is more severe when the ground is unfrozen under the snow during the winter months. Doubtless the fungus is more active under those conditions than when the ground is frozen before snow falls.

dence, however, was inconclusive and until more careful study can be made of these phenomena no definite conclusions can be drawn.

Because the complete life cycles have not been worked out satisfactorily it is impossible to definitely classify the organisms. However, the cultures sent from Japan by Tasugi, which he classifies as *Typhula graminum* Karst., appear to be similar to those in group III.

PATHOGENICITY TESTS

Pathogenicity tests of the organisms on plants grown in the greenhouse were unsuccessful. This probably was due to the fact that the temperatures in the greenhouse were too high for infection to cause any material injury. Wheat was sown in sterilized soil in pots and placed in the ice chest after having been surface inoculated with sclerotia produced from culture No. 226. Browning of the leaves and stems occurred and evidence of hyphae among the tissue was present but no sclerotia formed. However, successful inoculation was obtained with Nos. 282 and 285. Sterilized kernels of Turkey Red wheat were planted in sterilized Ottawa silica sand, which was kept moist with Sach's nutrient solution throughout the experiment. One week later sclerotia from cultures 282 and 285 were placed on the sand. Large test-tubes 12 inches long, containing sand and the wheat plants, were placed in the dark in refrigerators at temperatures just above 0° C. In one location the test tubes were subjected to occasional temperatures below 0° C. so that ice formed periodically around the plants. It was in these test-tube cultures that the most successful infection was obtained. Mycelial growth occurred up the stems of the wheat plants and sclerotia were formed embedded in the tissues and along the roots. (Fig. 4.) Reisolations of the two organisms were made from both sclerotia and infected tissue. It was possible also to inoculate plants with sclerotia produced in the field. Soil containing sclerotia was obtained from infested plots at Sandpoint and allowed to remain under outdoor conditions during the summer. Turkey Red wheat was planted in some of this soil that had been brought to the laboratory in the fall. The soil was put in glass tumblers and placed in a refrigerator at a temperature of about 0° C. It was kept moist with Sach's nutrient solution. Sixty days later one plant out of three inoculated was killed; mycelial growth was profuse up the stems and over the surface of the soil. (Fig. 4, B.) Sclerotial formation was abundant under the leaf sheaths. The organism was reisolated from the sclerotia and diseased tissue.

Pathogenicity tests have also been successful when performed in the field. Sclerotia from several different cultures were placed outdoors in the soil early in the summer of 1931. Kharkof and Turkey Red wheat was planted early in the fall where the sclerotia had been all summer. The following spring, 1932, from 50 to 100 per cent of the wheat was killed

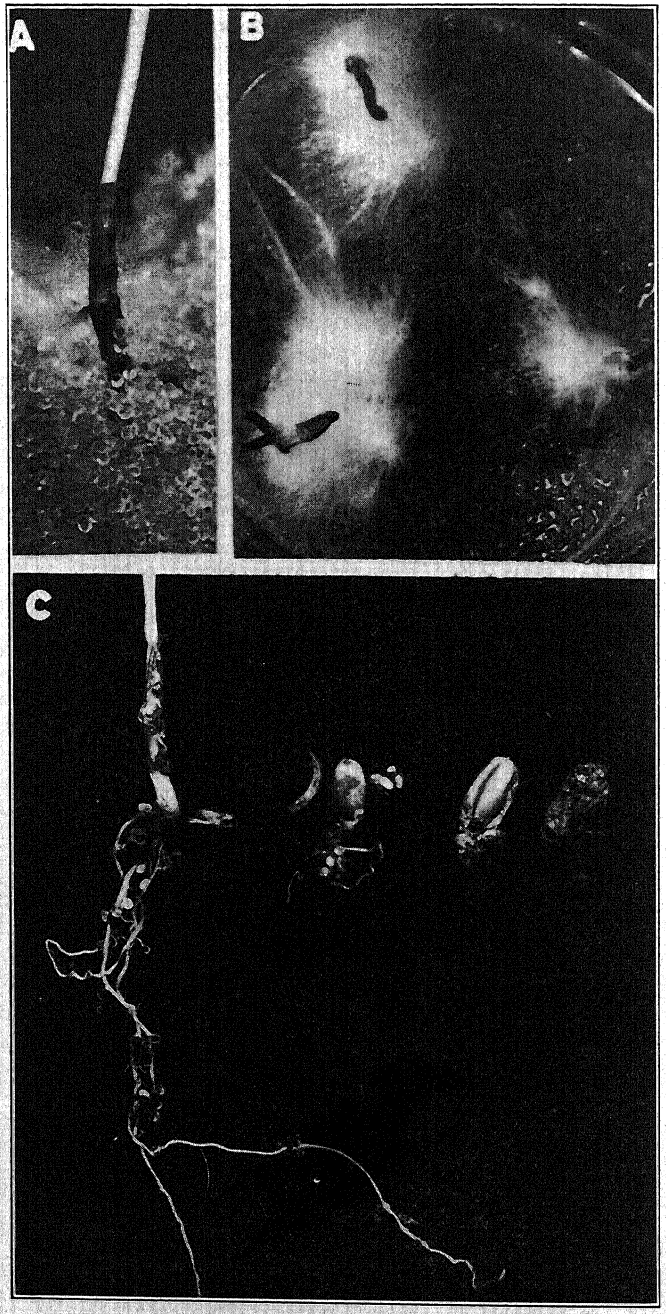


FIG. 4. A. Wheat plant inoculated with culture No. 282 growing in sterilized Ottawa silica sand. Note sclerotia embedded in the leaf sheath of the plant. B. Three wheat plants growing in soil containing sclerotia and secured from badly diseased wheat plots at Sandpoint, Idaho. Wheat was planted in this soil after it was stored out of doors during the summer. Note the mycelia on the roots. B. On the left is wheat plant shown in A after removal from the sand. Sclerotia are embedded in the leaf sheath and attached to the roots. The three seedlings on the right were killed at germination.

where planted over some of the cultures. (Fig. 2.) Culture 226 caused partial killing of plants, while cultures 277, 278, 279 killed all plants outright. The symptoms produced were identical with those found originally in the fields. The other cultures produced no symptoms.

Superintendent J. H. Christ of the University of Idaho Substation Farm at Sandpoint conducted an experiment in 1932 designed to test the susceptibility to the snow-scald fungus of the various winter barleys grown on the Substation Farm. After the various barley varieties were planted, soil was gathered from areas where the disease had been especially severe the year before and was scattered broadcast over the barley plots. A snow fence was constructed in order to keep the snow on the inoculated areas. As a result of the inoculation, the barley plots where the soil was applied were entirely killed, while the noninoculated plots, although very severely damaged, were not entirely destroyed. Evidence so far would indicate that there is considerable difference in susceptibility among the various winter wheats grown on the Sandpoint Substation Farm. As indicated above, however, none of the barleys grown possessed sufficient resistance to withstand the heavy inoculation given them, although the noninoculated seedlings showed a varietal resistance.

CONTROL

Würtenberger (16) found that applying sodium nitrate, leuna saltpetre, or sulphate of ammonia to the surface of the soil followed by thorough harrowing would keep the fungus under control. Bokura (1) recommended spraying with Bordeaux mixture just before snowfall. He found that other fungicides, such as copper-soap solution and lime-sulphur mixture, were also effective. He observed that grain sowed either early or late was particularly badly infected, but that resistant varieties could be safely sown at any time. Crop rotation is suggested by Müller (6).

Experience has shown that in the high-altitude regions of Teton Basin, Idaho, early-seeded wheat is more severely injured than that sown later in the season. A fair stand may be secured even in epiphytotic years when wheat is seeded fairly late.

SUMMARY

Diseases of grain and grasses produced by several sclerotial fungi that appear in the field after the snow melts have been increasing in occurrence and importance in the high-altitude regions in Idaho during the last few years. Fourteen cultures have been obtained of these organisms whose optimum temperatures for growth range from 0° to 10° C. Although attempts have been made to obtain the perfect stage, these have not been successful. These fourteen fungi have been placed in four groups according to their morphological and physiological characteristics. One of these

groups corresponds in growth characteristics with a culture of *Typhula graminum*, supplied by H. Tasugi, of Japan. Pathogenicity of several of these organisms has been established both by laboratory and field inoculations. The disease is best controlled by late seeding and also by using resistant varieties of cereals.

DEPARTMENT OF PLANT PATHOLOGY,
AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF IDAHO,
MOSCOW, IDAHO.

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BLACK ROT OF COFFEE IN MYSORE

M. J. NARASIMHAN[1]

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Coffee diseases, similar to the "black rot," or "koleroga" as it is called in Mysore, have been known to occur in various tropical countries, such as Venezuela, Jamaica, New Caledonia, the Belgian Congo, Puerto Rico, and Guatemala. The symptoms of the disease occurring in Mysore, the conditions under which the causal fungus thrives, and a description of the fungus have been given by Coleman, Venkata Rau, and Narasimhan (6) and by Butler (5).

The fungus grows on the dorsal surface of the leaves and over the berries in the form of a web or pellicle, which is shiny gray when dry and can be easily peeled off when wet. On the petioles, and to some extent on the twigs, it can be traced, extending as a thin ribbon. The affected leaves and berries turn brownish at first and, later, black. The blackened leaves hang

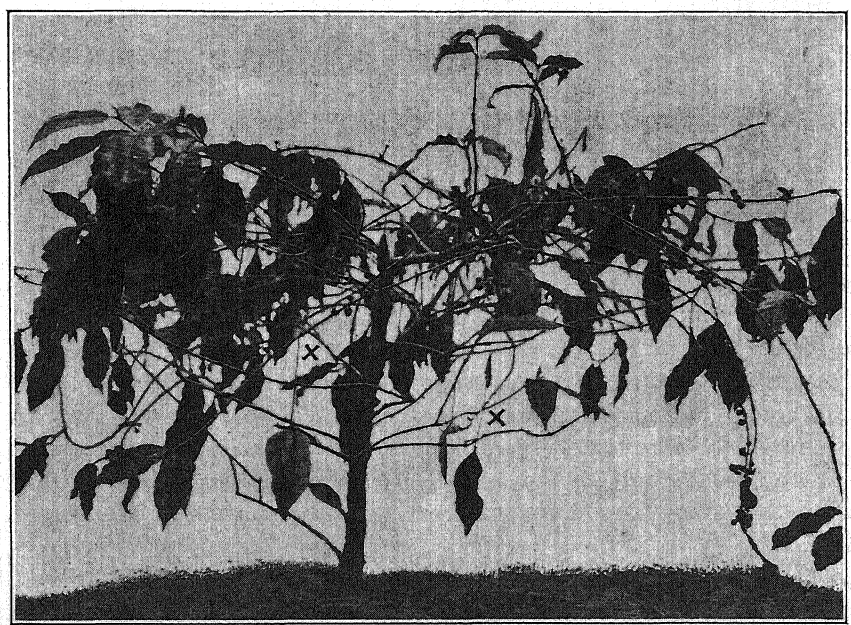


FIG. 1. Coffee bush attacked by *Corticium koleroga* showing some of the leaves suspended by mycelial strands at X.

[1] The writer wishes to acknowledge his indebtedness to Dr. Leslie C. Coleman, Director of Agriculture in Mysore, for the helpful suggestions given in the course of the investigation, which was carried on at the Coffee Experimental Station, Balehonnur.

suspended on the tree by means of the fungus strands connecting the petiole and the stem (Fig. 1). A brownish juice oozes from the leaves after decomposition. In attempting to determine the cause of this blackening, interesting observations on the life history of this fungus were made and are here presented.

Generally considered, it has to be admitted that investigations on this type of disease, which has been reported on coffee in various parts of the world, have not been complete, with the result that varying opinions have been expressed by investigators as to the identity of the fungus and the course of the disease.

Cooke (7) first named it *Pellicularia koleroga* and illustrated a type of spore that Fawcett (9) proved to belong to another fungus associated with the disease; Ernst (8) examining the Venezuelan specimen, named the fungus *Erysiphe scandens,* owing to its superficial nature. Von Höhnel (11), though he had only herbarium specimens available for examination, gave an accurate description of the basidia and basidiospores formed by the fungus and named it *Corticium koleroga.* These basidiospores, which are easily dislodged from the sterigmata, were first illustrated by photomicrographs by Coleman *et al.* (6).

MATERIAL AND METHODS

Affected coffee leaves were collected during the heavy monsoon, showing different stages of the disease, viz., leaves with grayish white film, those showing partial browning, and those that have become entirely black. The materials were fixed in Bouin's fixing solution. At first a difficulty was encountered, viz., that the fungus film soon separated from the leaf during the process of washing. This was obviated by dipping the material in melted ½ per cent agar before placing in the fixing fluid. For studying the fungus film itself, the following method was adopted:

A piece of affected coffee leaf was placed in water till the film, aided by careful manipulation with a needle, separated from the leaf and floated on the surface. It was drawn onto a slide, fixed on the slide itself for 24 hours in Bouin's fixing solution, and carefully washed in repeated changes of water. After the excess water was removed, 0.2 per cent celloidin in equal quantities of ether and alcohol was poured over it and allowed to dry. It was found that the film could, after this treatment, be safely stained in Heidenhain's iron-alum haematoxylin, dehydrated, and mounted in balsam.

MORPHOLOGY OF THE FUNGUS

The course of the disease can be divided into 2 distinct stages, viz., one in which a uniform white film is seen covering the dorsal surface of the leaf (Fig. 2, A), the other, in which hyphal clumps are found scattered all

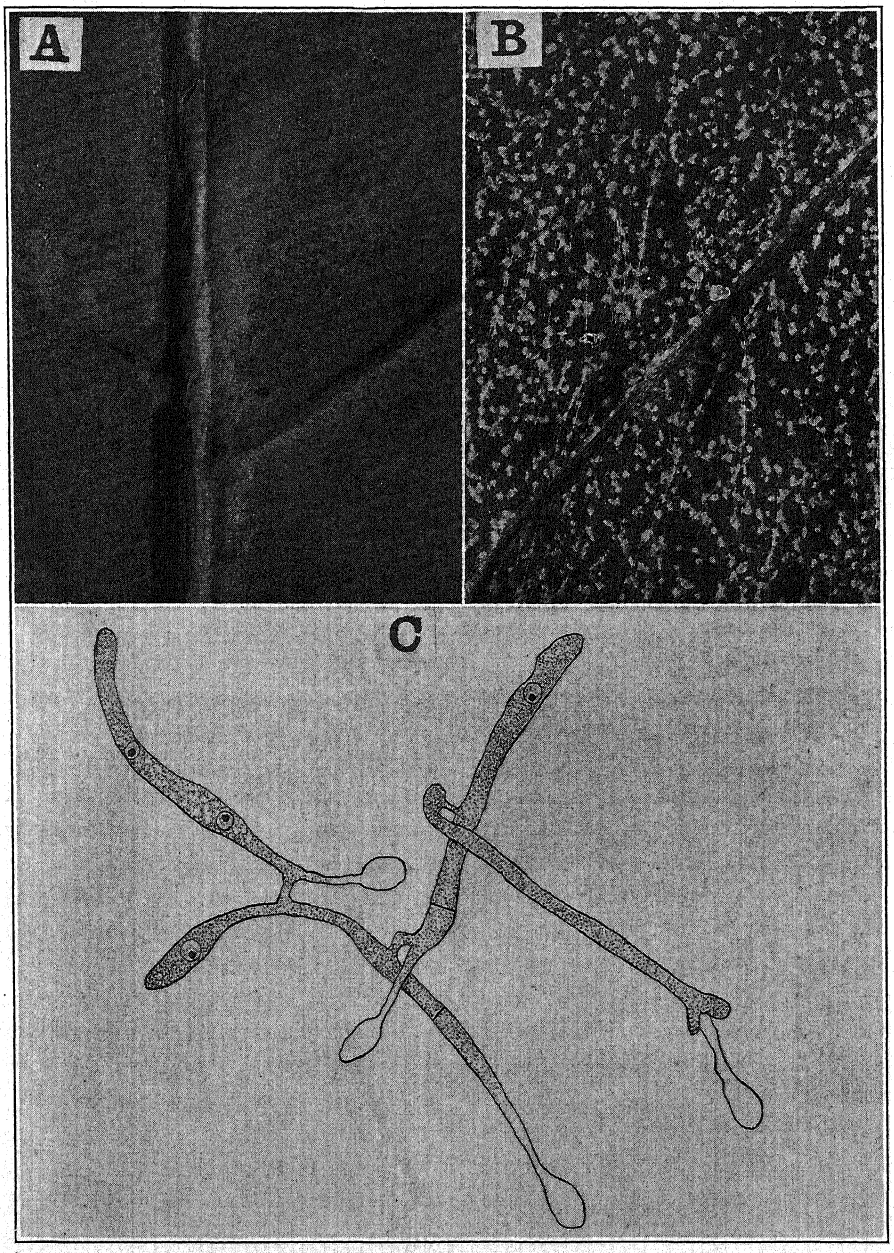


FIG. 2. Mycelium of *Corticium koleroga*. A and B. On diseased coffee leaves. A. Note the uniform whitish film on the dorsal surface. ×4. B. Note the hyphal clumps or sclerotia scattered with thin intervening spaces all over the lower surface. ×6. Photographed by Dr. Leslie C. Coleman. C. Anastomosis of hyphae from different spores.

over the affected leaf, the intervening mycelium being thin (Fig. 2, B). These may be differentiated as the early pellicle stage and the late sclerotial stage.

The Pellicle Stage. During the early stages of the disease, the film, when wet, can be peeled off the leaf, but it does not come off easily on areas where the leaf has already turned brown. It is made up of a mass of interwoven threads, "with here and there a primary strand consisting of a number of hyphae running parallel to each other" (6). The color of the mycelium is hyaline. No clamp connections or anchor cells, as in the case of "thread blights," have been found. Instead of these, repeated hyphal fusions have been found to be formed between the threads. One of 2 hyphae lying close together develops a lateral branch, which fuses with the other hypha and establishes a connection between them; or, where 2 hyphae cross, fusion by means of a short connecting branch occurs close to the angle formed by the 2 hyphae. Such connecting branches of 2 different hyphae have been found in *Panus stypticus* and *Coprinus lagopus* by Buller (3, p. 154) and named by him "bridging hyphae," and in *Hypochnus solani* by Müller (13).

The basidia are formed at the ends of lateral branches, being round or ovoid and measuring 8.5–12 μ in diameter, and are not found in clusters of 3 or 4, as Von Höhnel (11) reported. The basidiospores, borne on long tapering sterigmata, have been described and photographed by Coleman *et al.* (6). Fully developed spores from fresh material measured 9.1 μ × 3.4 μ. In no instance were they found to measure 13 μ in length, as reported by Burt (4). The length of the sterigmata was reported to be 2 μ by Von Höhnel (11) and 9–11 μ as measured on fresh material by Coleman *et al.* (6). It was found that the length of the sterigmata was inconstant, varying from 5 μ to 11.5 μ. Figure 3, B, shows short sterigmata bearing fully developed spores, while in figure 3, A, it is seen that the sterigmata bearing immature spores are long and tapering and of unequal lengths. The spores are easily dislodged and lie about in the meshes of the film. They are slightly flattened on one side, rounded at one end, and somewhat pointed at the other. Abundant spore formation has been noted in the estate only at the time when the humidity is high. The best method of studying the spores is the one followed by Coleman *et al.* (6), viz., attaching pieces of affected leaves bearing basidia to the inner surface of the Petri-dish cover and allowing the spores to drop on agar contained in the bottom dish. In heavy monsoon weather, the spores have been found to drop in 4 hours' time and germinate 2 hours later, forming germ tubes with swollen ends (Fig. 3, C).

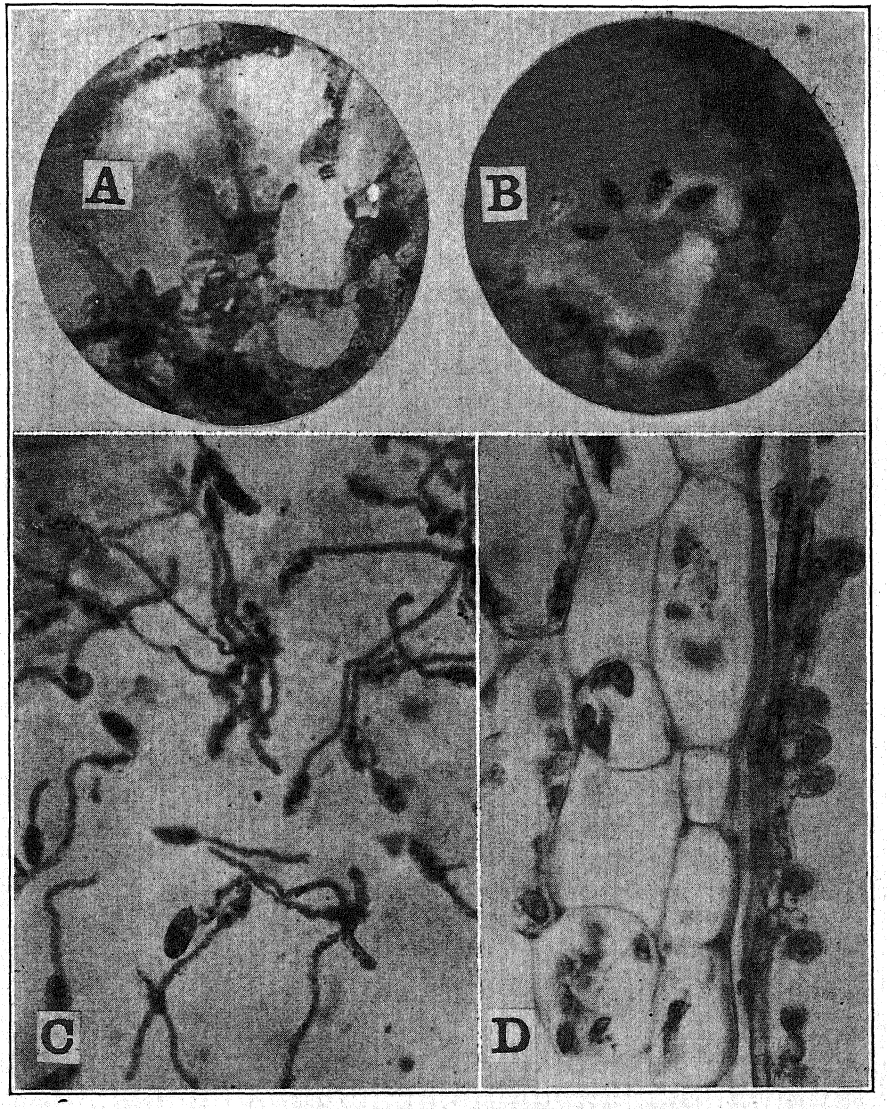


FIG. 3. A and B. Photomicrographs of basidia of *Corticium koleroga*. ×900. Note the immature basidiospores and tapering sterigmata of unequal lengths in A and the fully developed basidiospores and short and equal-lengthed sterigmata in B. C. Germinating basidiospores. ×1,350. D. Section of coffee leaf with fungus film on its lower surface. ×900. Note the nuclei in some of the basidia.

When a large number of spores germinate side by side on agar, anastomosis has been observed often between the hyphae formed by the germination of 2 different spores. Sometimes, adjacent threads developing from

2 different spores fuse, at others the threads from 2 spores lying far apart. This has not been observed to take place during the early stages of germination but only after the germ tubes have grown to some extent. Both terminal and lateral fusions have been observed (Fig. 2, C). Müller (13) reported similar hyphal fusions of germinating spores in *Hypochnus solani*. Buller (3) reported the occurrence of such hyphal fusions of germinating spores not only in the Hymenomycetes but in some of the Ascomycetes, viz., *Trichoderma lignorum, Pleurage anserina,* and *Ascobolus magnificus,* and in one of the Fungi Imperfecti, viz., *Colletotrichum trichellum*. The significance of these hyphal fusions, as Buller pointed out, lies in the fact that the conduction of food materials to the sclerotia and fruit bodies is facilitated and that the association of nuclei in conjugate pairs is thus made possible.

The spores as well as the hyphae resulting from germination are uninucleate. After fusion, the hyphae, which develop further, are always binucleate. Similar fusions leading to the binucleate stage have been reported by Willy Harnack (10) in the case of *Schizophyllum commune* Fr. and *Collybia tuberosa* Bull.

Isolation of the Fungus. Spores were made to drop on onion agar, following the method of Coleman *et al.* (6). These subsequently developed into a hyphal mat on the surface of the agar, which was transferred to tubes of various media. On oat agar and onion agar whitish aerial mycelium is produced, but on peptone sugar agar and peptone starch agar only a surface growth develops. A fairly good growth is obtained on bits of filter paper moistened with peptone starch solution recommended by Müller (13) for *Hypochnus solani*. The development of basidia has never been noted on this medium, as claimed by Müller for *H. solani*. No fructifications have ever been noted to develop on any of the media tried hitherto.

Following Bavendamm's (2) cultural experiments on wood-rotting fungi, the effect of adding to the media varying quantities of tannic acid, gallic acid, pyrogallol, and guajacol, on the growth of *Corticium koleroga* was noted. It was found that on onion agar containing 0.1 per cent tannic acid or gallic acid a brown halo was formed in advance of the growing mycelium (Fig. 4, A). On media containing 0.05 per cent guajacol, a dark reddish brown halo (Ridgway's Pompeian Red) was formed (Fig. 4, B).

The formation of a brown halo on media containing tannic acid was found by Bavendamm to be characteristic of lignin-destroying organisms, such as *Stereum purpureum* and *Trametes radiciperda*. He found that those involved in cellulose disintegration, such as *Merulius lacrymans* and *Coniophora cerebella,* did not form a brown halo. The brown discoloration, according to Bavendamm, is caused by the action of the oxidase produced

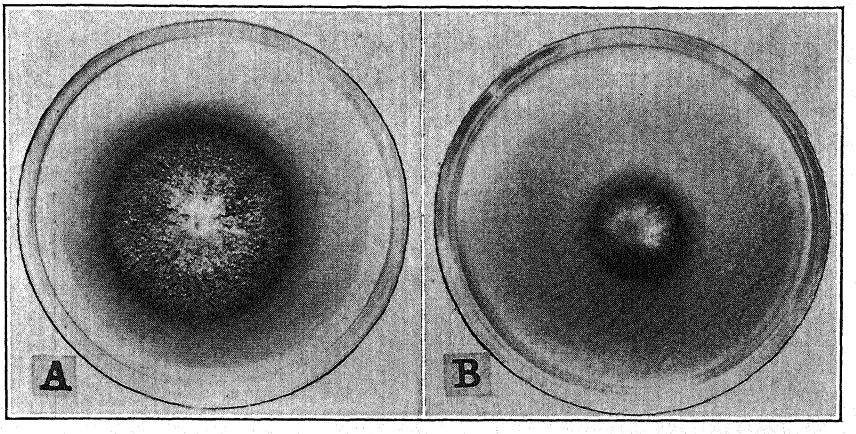


FIG. 4. Pure 6-day-old cultures of *Corticium koleroga*. A. On onion agar to which 0.1 per cent tannic acid had been added. Note the dark halo around the colony. B. On onion agar to which 0.05 per cent guajacol had been added. The dark-red halo around the colony appears black in the photograph.

TABLE 1.—*The cultural behavior of Corticium koleroga on onion agar to which various chemicals were added*

Chemical	Percentage of chemical added to the media			
	0.05%	0.1%	0.25%	0.5%
Tannic acid	Very good growth Brown halo	Very good growth Brown halo	Fair Brown halo	Poor Brown halo
Gallic acid	Good growth Brown halo	Poor Brown halo	Nil	Nil
Pyrogallol	Poor growth Brown halo	Nil	"	"
Guajacol	Fair Reddish brown halo	"	"	"

by the fungus on tannin. In cultures of *Corticium koleroga* there are definite indications of the presence of oxidase.

The Sclerotial Stage. The fluffy, pulverulent appearance of the film in course of development changes into one that is dotted with a large number of hyphal clumps, the intervening spaces being thin (Fig. 2, B). At this stage the mycelium gradually changes from a whitish to fuscous color and the affected leaf turns to a rusty brown. These sclerotia are formed by

repeated branchings of short cells and are ultimately pseudoparenchymatous in nature, the component parts consisting of short binucleate cells (Fig. 5, A). The sclerotia are hyaline during the early stages but change to a fuscous brown later. Neither Von Höhnel (11) nor Burt (4) found these structures in specimens of *Corticium koleroga* examined by them. Burt

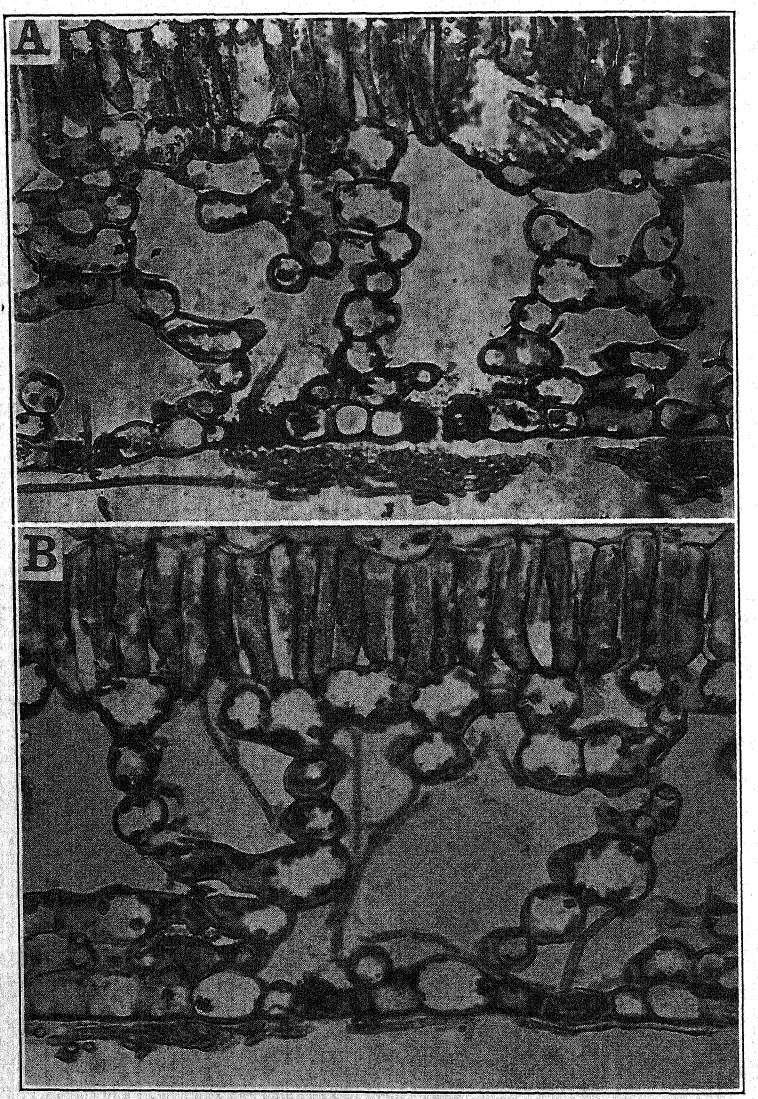


FIG. 5. Sections of coffee leaves infected with *Corticium koleroga*. ×300. In A, note the sclerotia and the penetration of fungus through the stomata. In B, note the intercellular mycelium.

(4), however, noted their presence in *C. Stevensii,* parasitic on leaves and branches of apple, pear, and quince. Wolf and Bach (14) found these structures sparsely on grapefruits affected by thread blight and in abundance on affected pears, apples, pecans, and pomegranates. These were first reported in *C. koleroga* on coffee by Coleman *et al.* (6). The sclerotia carry the fungus through the dry season.

From descriptions given in literature of similar coffee diseases in the tropics, one is tempted to conclude that these structures are characteristic of the disease. Kuyper (12), describing the "*Zilverdraadziekte*" of coffee in Surinam, reported on the occurrence of "Hyphen Kluwens" peculiar to this disease. Bally (1) compares these with the hyphal-clump stage ("*Knobbeltjesvorm*") in the disease known as *Djamoer Oepas* on coffee in Java. In his comparative study of the coffee diseases occurring in Mysore, Puerto Rico, and Venezuela, Fawcett (9), who did not recognize the basidiomycetous nature of the fungus, described and illustrated structures that he called holdfast cells. Burt (4) gave his opinion that these were probably young basidia. The writer is inclined to believe, judging from the figures, that they are early stages of sclerotia. The small hold-fast cells, which, according to Fawcett, differentiate the candellilo disease of coffee in Venezuela from other diseases of similar nature are comparable to the tiny clumps of sclerotia found during the early stages in Mysore.

PENETRATION OF THE FUNGUS INTO THE COFFEE LEAF

The question whether this type of fungus penetrates the tissues has not been satisfactorily investigated. Von Höhnel (11), who examined the specimens at Kew, reported that the fungus was entirely superficial. Burt (4) who based his comprehensive description of *Corticium koleroga* on a small portion of the Venezuelan specimen and microscopic preparations of the Mysore specimens sent to him from Kew, reported the presence of intercellular vegetative mycelium. He could not have examined affected tissues, as, from his own words, only preparations of the basidiospores and hyphae were sent to him from Kew. Coleman *et al.* (6) found no evidence of penetration. They suggested that the death of the leaves, as evidenced by the blackening, might be due partly to reduced respiratory activity owing to blocking of the stomata and partly to the secretion of an enzyme or toxin. Butler (5) thought it probable that there was a direct absorption of nutrition by the parasite by osmosis from the leaf. Thus, it will be seen that varying opinions have been held as to whether or not the fungus penetrates the tissues. In order to settle this question the writer examined a large number of hand and microtome sections of affected leaves in various stages of the disease.

Leaves covered on the dorsal surface with the uniform whitish film, *i.e.*, in the early stage when basidia were formed, have never shown the presence of intercellular mycelium in the tissues (Fig. 8). No trace of any sort of injury to the epidermis can be noted. Any evidence of penetration into the tissues can be made out only during the later stage, when hyphal clumps are formed. Sections of leaf areas that have turned rusty brown, show, attached to the lower epidermis, compact masses of pseudoparenchymatous cells (Fig. 5, A).

The hyphae emerging from these masses penetrate the tissue through the stomata (Fig. 5) and permeate the spongy parenchyma, often reaching as far as the palisade cells. In leaf areas that are adjacent to a brown patch and still green, the formation of the sclerotia is to be seen, with occasional penetration of the tissues. The brown color appears in the leaf only after penetration of the tissues by the hyphae. The secretion of oxidase by the fungus that penetrates the tissues of the leaf perhaps causes the blackening of the leaf. During later stages of the disease, the leaves begin to decompose because of invasion by bacteria, and a brownish liquid oozes out.

It is often seen that during very heavy rains some of the leaves turn black, though no fungus is noticeable on them. This is due to the fact that the leaves get water-soaked and become decomposed by bacteria.

COMPARISON WITH SIMILAR DISEASES OCCURRING IN OTHER PARTS OF THE WORLD

Our imperfect knowledge of the complete life history of this type of disease on coffee, occurring in various parts of the tropics, led investigators to believe that some of these are different from the type occurring in Mysore. Fawcett (9) found that the Mysore and Puerto Rican diseases were similar but different from the Venezuelan disease.

Reviewing our present state of knowledge of these diseases, Bally (1) says that the question "Whether the spinnewebeziekte of Java, the Zilverdraadziekte of Surinam, the candellilo of Venezuela, the koleroga of India, and perhaps a disease which Bernard in 1907 described on tea, are caused by the same fungus, remains to be settled." Of these, the Java disease is said to be different, owing to the presence of anchor cells, and is classified under the Marasmioideae group; but, as Bally pointed out, the disease on tea described by Bernard and *Corticium koleroga* on coffee are almost identical, except for the light pink color of the former.

He however expressed the opinion that the *zilverdraadziekte* of South America is not identical with any of the described kinds found in the East, as it differs from *Corticium koleroga* in that its mycelium enters by way of the stomata and develops hyphal clumps.

In view of the writer's finding in the Mysore disease that the sclerotia-forming mycelium penetrates the leaf tissues, there seems to be no ground

for considering the South American disease different. Very little is known of the life history of the candellilo disease of Venezuela. Fawcett (9) considered it to be different from the Mysore and Puerto Rican diseases, because of the smaller holdfasts. From an examination of the figures given by him, it may be concluded that these holdfasts are probably early stages of sclerotial formation. When more is known about this disease, it will probably be found that it is identical with the Mysore disease.

The facts presented above are, in some important respects, at variance with those presented by Burt (4) in his description of *Corticium koleroga*. He pictured the course of the disease as follows:

"The parasitic vegetative mycelium forms long slender mycelial strands of rather uniform diameter, whitish or pallid at first, finally fuscous, running along the branches and midrib and veins of the leaves, infecting the leaves, and ramifying between the cells of the leaf-parenchyma, *finally*[2] emerging at many points on the under side of the leaf to form minute fructifications, soon laterally confluent into a thin arachnoid, perforate membrane covering the under surface of the leaf between the midrib and the principal veins."

The above description leads one to believe that the penetration of the tissues by the fungus takes place prior to the formation of the basidia and the basidiospores. Penetration by the hyphae that bear basidia has never been observed by any of the investigators. As seen in figure 3, D, the fungus at this stage is entirely superficial. It is only later, when it forms the sclerotia, that penetration is in evidence. Basidia have never been observed to form after the fungus penetrates the tissues. As to what the fungus feeds on during the pellicle stage it is difficult to say unless it is assumed that there is absorption of nutrition by osmosis.

Control Measures. The disease has been satisfactorily controlled by spraying with Bordeaux mixture (5:5:50) before the outbreak of the monsoon. The use of an adhesive, such as lime caseinate, has been found to be essential in order to prevent the mixture from being washed off by the heavy rains. An area of 12,000 acres of coffee was sprayed during the year in Mysore.

SUMMARY

Corticium koleroga (Cooke) Von Höhn. causing black rot of coffee in Mysore, has two distinct developmental stages, viz., the early pellicle stage in which a white uniform film is seen covering the under side of the leaf and the late sclerotial stage in which sclerotia are found scattered all over the dorsal surface.

[2] Italicized by the writer.

Basidia and basidiospores are to be observed only during the pellicle stage of development. The fungus is entirely superficial at this stage. Bridging hyphae are commonly seen.

Anastomoses of the hyphae formed by the germination of the basidiospores have been observed.

By adopting Bavendamm's cultural method it has been found that *C. koleroga* produces oxidase during growth.

Penetration of the leaf through the stomata by the fungus has been observed only during the late sclerotial stage. The blackening of the leaf is considered due to the secretion of oxidase into the tissue by the fungus.

A comparison between the black rot of coffee in Mysore and similar diseases on coffee occurring in other parts of the world shows that there is not sufficient ground for considering any of them different except the thread blight occurring in Java.

DEPARTMENT OF AGRICULTURE,
MYSORE STATE,
INDIA.

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A MOSAIC OF THE FIG IN CALIFORNIA[1]

IRA J. CONDIT AND W. T. HORNE

(Accepted for publication Dec. 27, 1932)

The foliage of fig trees in California is, in general, free from serious diseases such as leaf rust, *Kuehneola fici* (Arth.) Butler, which is an important factor in the commercial production of figs in many other districts. There has long been recognized here, however, a mosaic-like trouble of fig leaves, which occurs on practically all varieties wherever grown in this State. It appears to have been almost totally overlooked in the pathological literature of the fig both in this and in foreign countries. This leaf trouble is sufficiently severe in some cases to cause not only leaf drop but fruit drop, as well, and, therefore, warrants consideration from various standpoints such as variety resistance or immunity, possible insect vectors, and breeding of resistant types.

Fig diseases have been discussed by various writers ever since the time of Theophrastus (7, Vol. I, p. 401) third century B. C., who mentions a "scorching" of branches and foliage due to cold winds. Pellicano (3) describes a leaf scald of fig trees in Italy in which leaves turn yellow and dry up prematurely on account of drought, as well as chlorosis of foliage due to obscure causes. Vallese (8) in his book "Il Fico" discusses a chlorosis or yellowing of fig leaves and suggests that sprinkling with a weak solution of iron sulphate may produce beneficial results.

A leaf scald of Sicilian fig trees is mentioned by Portale (4) as being similar to the "squalor malorum" of Theophrastus. A light yellowing of the leaves is first observed, which is soon intensified even in the fruit, ending later with the shrivelling and dropping of both leaves and fruit. It appears to be due, according to Portale, to prolonged summer heat. L. Savastano (5) gives a brief account of a chlorosis of fig leaves in August following drought, the chlorosis being diffused over the leaf surface and not localized in spots.

None of these references to fig culture in Italy, however, describe any leaf troubles that produce mosaic-like spotting of the foliage.

A single reference to mosaic of fig leaves has been found in American literature, that of Swingle (6), who states:

"Many years ago I secured at Biskra, in the great date oasis of the Algerian Sahara, cuttings of a fig which has proven to be very well adapted to hot, dry, irrigated valleys in the Southwest. It is, so far as I know, the

[1] Paper No. 291, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

only fig among twenty-five or more varieties tested in the hot irrigated valleys where dates can be grown successfully, which does not show more or less severe mottling. It seems to be quite unaffected by the intensely hot weather in midsummer, the leaves remaining vivid green instead of being yellowed by mosaic mottling."

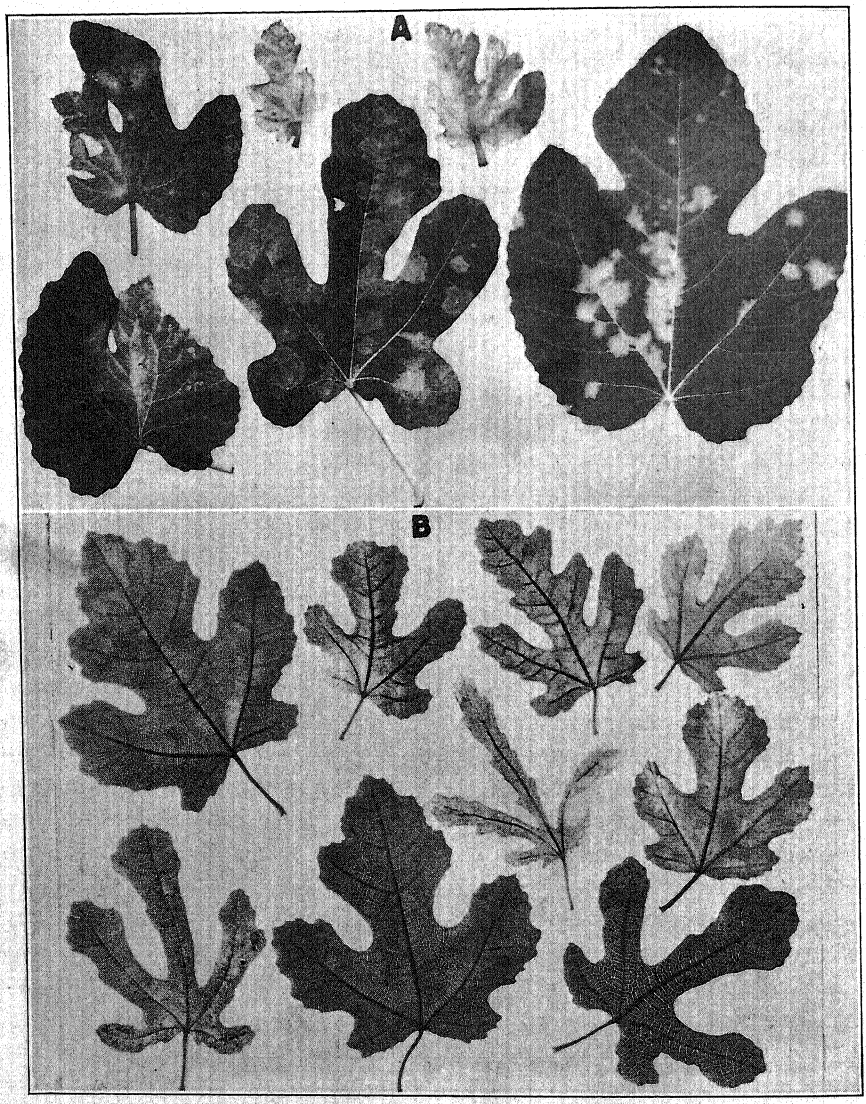


FIG. 1. A. Fig mosaic manifests itself not only as distinct yellowish spots on the normally green leaves but also in various malformations of the leaves. B. Budlings from healthy seedlings, growing on mosaic-infected trees, produce leaves with typical mosaic spots and malformations.

Hodgson has recently (2) reported that a mosaic of fig leaves is widespread in Tunisia but is of no economic importance.

MOSAIC SYMPTOMS

Fig mosaic manifests itself both on the leaves and on the fruit. On the leaves the mosaic spots are sometimes sharply delineated, the light yellow color of the affected areas contrasting strongly with the normal green color of the foliage (Fig. 1, A). The borders of mosaic spots are, however, usually indefinite, their light color blending gradually with the dark green of healthy tissue (Fig. 2, A). In some leaves the spots are of more or less uniform size, small, and densely scattered over the surface. In other cases they are of various sizes, of indefinite outline and appearing as irregular patches of light green, diffused widely throughout the blade of the leaf with no relation whatever to the position of the leaf veins. Mosaic spots often are bordered by a rust-colored band, evidently caused by the death of epidermal or subepidermal cells. Seldom does the mosaic bring about an actual necrosis or dying out of whole areas of leaf tissue.

One manifestation of fig mosaic, which appears to be systemic, is the malformation of leaves. In some cases certain twigs bear none but malformed leaves. In other cases both malformed and normal leaves are found on the same twig. Malformed leaves show an infinite variety of shapes and sizes (Fig. 3, B).

The appearance of mosaic spots on the fruit is very similar to that on the leaves (Fig. 2, B). The premature dropping of figs appears at times to be due to the presence of these mosaic spots on the surface of the fruit and on adjacent leaves.

VARIETY SUSCEPTIBILITY

In the commercial fig districts of California mosaic apparently is not sufficiently serious to alarm the growers. Of the 5 leading commercial varieties the Kadota and Calimyrna are least affected by mosaic, the deep green foliage showing little if any mottling. The White Adriatic and the Brown Turkey, under good cultural conditions, are apparently little affected, although the presence of deformed leaves due to mosaic is common on trees of the former variety. The foliage of Mission trees is quite commonly seriously mottled by mosaic, often sufficiently so to interfere with photosynthesis and later to cause leaf drop; its presence on the figs may result in improper coloring of the fruit in drying.

Among the hundred or more varieties of figs in a variety planting at the University of California Citrus Experiment Station, Riverside, there are striking variations in the degree of susceptibility to mosaic. For convenience the varieties can be classified into 4 groups, depending upon the susceptibility

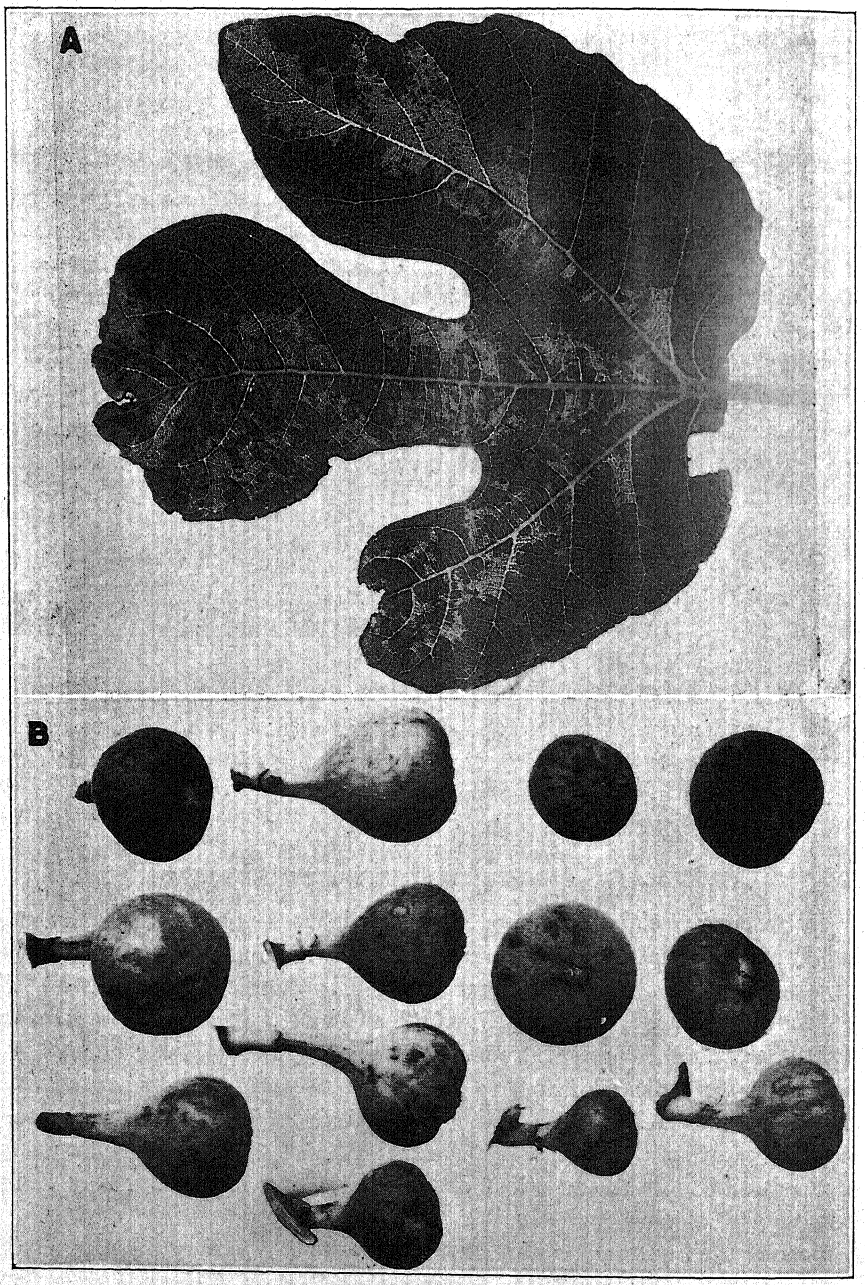


FIG. 2. A. In this fig leaf the mosaic patches are rather indefinite and not sharply delineated. B. Fig fruits, as well as leaves, show mosaic spots.

of leaves and fruit to this trouble; those immune or essentially so, those very slightly affected, those slightly affected, those appreciably affected.

There is only one variety that can be called almost immune from mosaic—an entire-leaf caprifig form of *Ficus palmata* Forsok. A careful

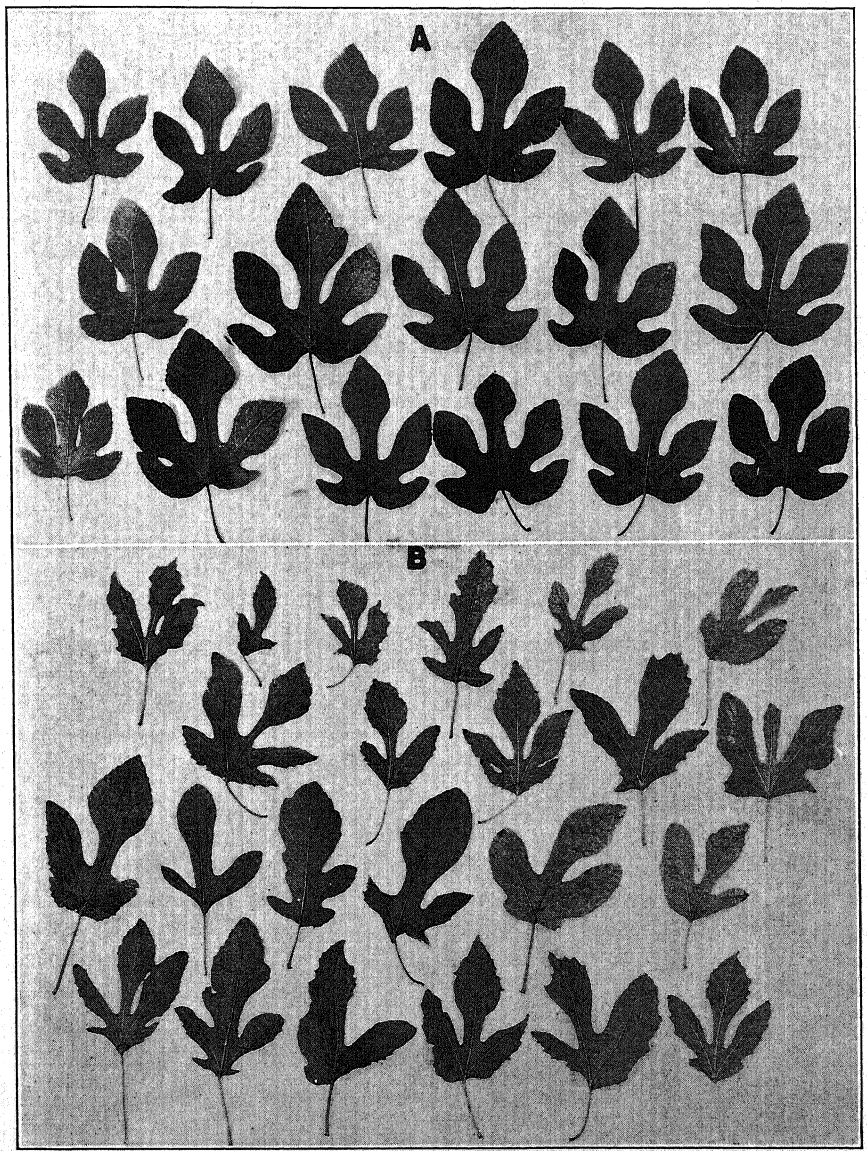


FIG. 3. Leaves from same Adriatic tree. A. Normal. B. Malformations due to mosaic.

search of 2 vigorous trees failed to show any leaves certainly affected by mosaic, although there were indications of its presence on a few leaves. Some seedling fig trees having *F. palmata* as the male parent appear to show immunity from mosaic.

In group 2 there are at least 4 varieties, very slightly affected by mosaic. The Biskra is a very vigorous tree, with the largest leaves of any variety studied, but occasional leaves show unmistakable mosaic spots.

The Hamma, which appears to be a form of *Ficus palmata* bearing edible fruit, is very slightly affected, but shows some indistinct mosaic areas in the leaves. A lobed-leaf caprifig form of *F. palmata* is affected about the same as the Hamma. Of all the varieties of common figs in the plot the Pastiliere is the least affected, only scattered spots appearing on fruit or leaves.

The Calimyrna, Kadota, Kassaba, White San Pedro, and Roeding No. 1 caprifig are varieties showing very apparent mosaic spots, but the color of the foliage is not sufficiently affected to materially reduce photosynthetic activity. At least, there is no apparent lack of tree vigor traceable to the presence of leaf mosaic. The Brunswick (Magnolia) foliage is slightly affected. Some cases are found where the immature Brunswick fruit itself is affected sufficiently to account for its premature abscission. In these cases the variety would logically fall into the next group.

The number of varieties very appreciably affected by leaf mosaic is large. No attempt is made here to list or describe all those represented in the collection but simply to point out some that appear especially susceptible. Considerable malformation of leaves is present in one caprifig tree of *Ficus pseudocarica* Miq., the malformed leaves, however, being restricted to the least vigorous branches.

Mosaic spots are very prevalent on both leaves and fruit of the White Ischia and the Celeste varieties, the latter being sufficiently affected to account for the premature shedding of fruit. The Cheker Injir, a Smyrna type fig, is very susceptible, the surface of the fruit being spotted with light green areoles, which later become rusty.

Some varieties, such as Baalie and Panache, show numerous leaves malformed by mosaic, the malformations in some cases dwarfing the entire leaf. Malformed leaves are especially common on Roeding No. 3 caprifig. De Jerusalem leaves have very pronounced and striking mosaic spots, which early develop necrosis; the immature figs are also markedly affected. In the White Adriatic the mosaic spots are widely diffused over the leaf surface and the trouble appears to be sufficiently serious to account for the dropping of terminal leaves (Fig. 4, B). Deformation of small green figs of certain varieties, such as Sultane, is attributed to the presence of mosaic.

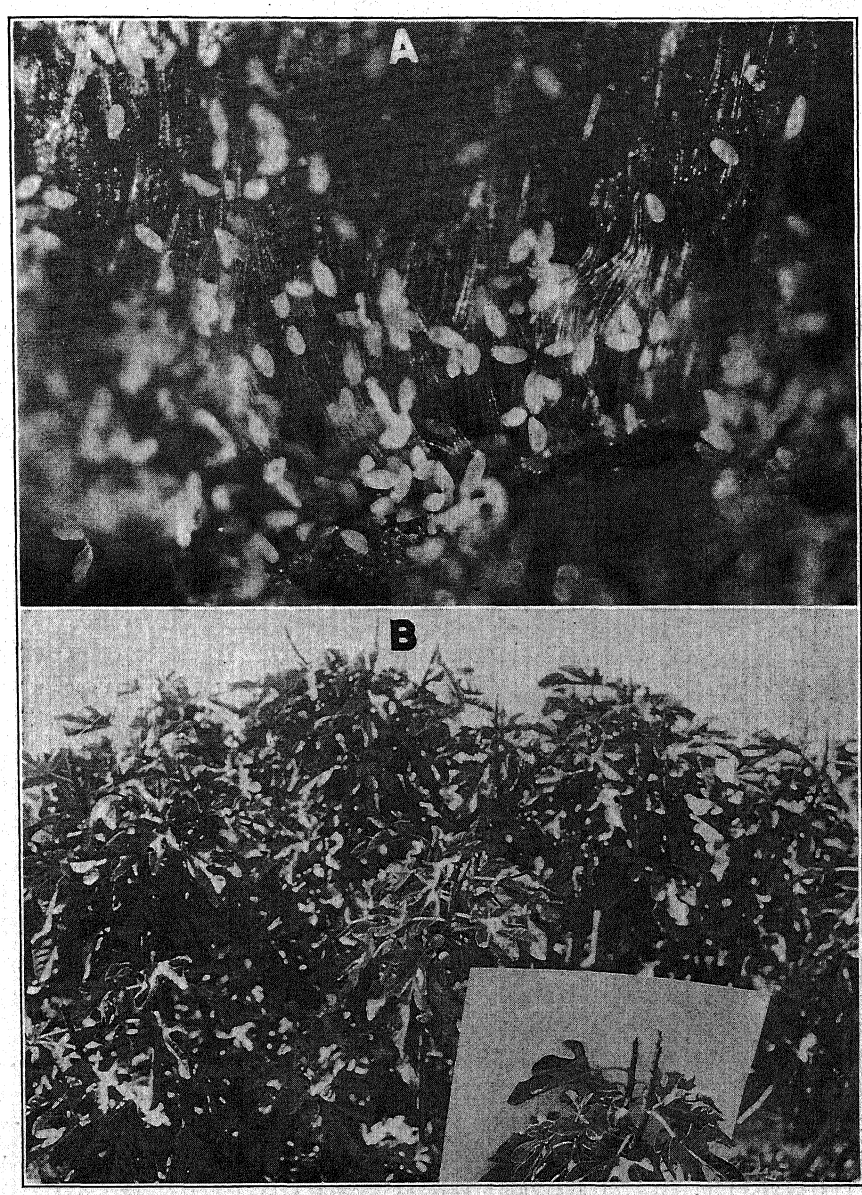


FIG. 4. A. Fig mites (*Eriophyes fici*) often infest bud scales and young leaves in immense numbers, sometimes causing the abscission of leaves and stunting of twigs. B. In some varieties, such as the Adriatic, fig mosaic appears to account for the early defoliation of terminal twigs.

POSSIBLE VECTORS

Insect pests are commonly suspected of causing malformations in leaves or of transmitting virus diseases when present. The foliage and branches of fig trees in California are not commonly infested with insect or other pests. The Mediterranean fig scale (*Lepidosaphes fici* Sign.) has become established in Fresno and Tulare Counties (California) and is rapidly spreading through commercial orchards. Certain species of thrips are at times found on the tender foliage but apparently do not injure the leaves to any appreciable extent. They are, however, often responsible for fungus infection of the fruit interior and for its ultimate spoilage (Hansen, 1). Aphid infestations of fig foliage are rare and evanescent and are seldom if ever injurious.

In 1920 an arachnid, *Eriophyes fici* Ewing, was found to be very prevalent in fruits and on terminal bud scales of figs at Fresno. This mite is now known to be present on fig trees in all parts of California and in some localities in Oregon. Infestations of terminal buds (Fig. 4, A) are often sufficiently serious to cause the drying up and dropping of very small immature leaves and the stunting of twigs. In a letter dated September 16, 1932, J. R. Watson reports that he has noticed fig mites in Florida and in one case the infestation was sufficiently severe to cause all leaves to drop from fig trees at Gainesville. In the fig districts of Mississippi, Louisiana, and Texas neither fig mites nor mosaic appears to attract attention, possibly because of heavy pruning and the resultant vigorous succulent growth. Under date of October 27, 1932, Dr. G. Grandi, Lab. di Entomologia, Bologna, writes that neither fig mites nor foliage mosaic of fig trees is mentioned in Italian literature.

Recent conferences with certain specialists fail to show any reference in literature to the transmission of a virus disease by mites. In the disease under discussion it is possible that the spots on fig leaves may result from the localized feeding of mites when the leaves were young and tender. In August, 1932, however, 3 fig trees, probably of the Mission variety, were seen near Ashland, Oregon, with mites abundant on the bud scales and very young leaves but with deep green foliage, apparently entirely free from mosaic. We have suspected fig mites of transmitting the virus of leaf mosaic, but thus far our suspicions are not confirmed or supported by experimental data.

FIELD AND LABORATORY STUDIES

The fig tree is almost universally propagated by means of cuttings from mature wood. Such cuttings would naturally reproduce any disease that

is systemic in the branches. In order to throw light on the question whether the mosaic-like lesions are local disturbances or stigmanoses in the leaves and fruit, or the results of some more general disturbance in the plant body, the following experiment was carried out: Twenty-five cuttings each were taken in March from 4 different varieties of fig trees known to be seriously infected with mosaic. These cuttings were rooted in sand in a closed box in the greenhouse and by May 5 many had leaves showing mosaic symptoms. On June 8 a census was taken of the 100 rooted cuttings with the result that 74 showed mosaic certainly present, 16 showed no positive indications of mosaic, while 10 had insufficient leaf growth to enable one to draw any conclusion. Of the 100 twigs left on the tree from which the cuttings were taken, 51 showed intense leaf mosaic on June 8, 30 showed mosaic to be certainly present, while 19 could not be located.

Seedling fig trees secured from seed of mosaic-affected parents have been grown in the greenhouse up to 12 inches in height without the appearance of any discoloration resembling mosaic on the foliage.

That mosaic is readily transmissible to healthy seedlings was strikingly shown by the following experiment: Two buds from each of 5 apparently healthy seedlings were inserted in July into branches of an orchard tree showing typical mosaic spots on the foliage. Nine budlings, alive and growing late in October, all showed unmistakable symptoms of leaf mosaic, evidently transmitted from the stock to the scion (Fig. 1, B). The plants from which the buds had been taken, kept in the greenhouse and lathhouse, put out new growth, which showed no symptoms of mosaic. Healthy seedlings, on which fresh mite-infested fig leaves were put, have, in 2 cases, at least, developed unmistakable mosaic spots. In this case the mosaic may have been transferred by contact infection but other attempts to transmit it in this way have all failed.

Studies of fig trees are being continued, but the preliminary results here reported are believed to constitute valid items of evidence tending to show that we have to do with a true mosaic disease.

UNIVERSITY OF CALIFORNIA,
CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

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BACTERIAL SPECK OF TOMATOES

MARY K. BRYAN

(Accepted for publication, Dec. 5, 1932)

INTRODUCTION

In the course of a study of bacterial spot of tomato (*Bacterium vesicatorium*) for a comparison of early symptoms on fruit with those caused by bacterial canker (*Aplanobacter michiganense*) some isolations gave pure cultures of a green fluorescent bacterium entirely different from either of the two organisms under investigation. Inoculations on young tomato fruits, made by swabbing with water suspensions of this third organism produced abundant spotting. Reisolations from the resultant infections gave the same green fluorescent organism as was used for inoculation, and this reisolation, in turn, produced the same small dark spots when swabbed on young fruits. It seems definitely established, therefore, that there is a third bacterial spot of tomato fruits heretofore confused with some stages of bacterial spot (*Bact. vesicatorium*). The name bacterial speck is proposed for this disease and the name *Bacterium punctulans,* n. sp., for the causal organism.

GEOGRAPHICAL DISTRIBUTION

Speck was first demonstrated as a new disease, as distinguished from bacterial spot, in the spring of 1931 at Bradenton, Florida. In 1930, however, the writer was shown a field in the same locality in which the larger part of the fruit was peppered with tiny black spots and no large, typical lesions due to *Bact. vesicatorium* were observed. It was supposed, however, that this was a phase of that disease, perhaps connected with sand injury. Specimens were collected and preserved but no isolations were made. Examination of these specimens in the light of present knowledge indicates that the disease was probably speck. From S. P. Doolittle it was learned that during more than one season this type of spot was more prevalent at Bradenton and did more damage than the typical spot caused by *Bact. vesicatorium.* He also observed the disease in considerable abundance in tomato plots at Madison, Wisconsin, in 1929.

Specimens infected with typical speck were also received in the summer of 1931 from Maryland. A green fluorescent organism, identical with that from Florida speck, was isolated from these and proved pathogenic.

SYMPTOMATOLOGY

The name *speck* was selected because of the minute nature of the lesions on the fruit, which seldom attain a diameter of more than 1 mm. (Fig. 1,

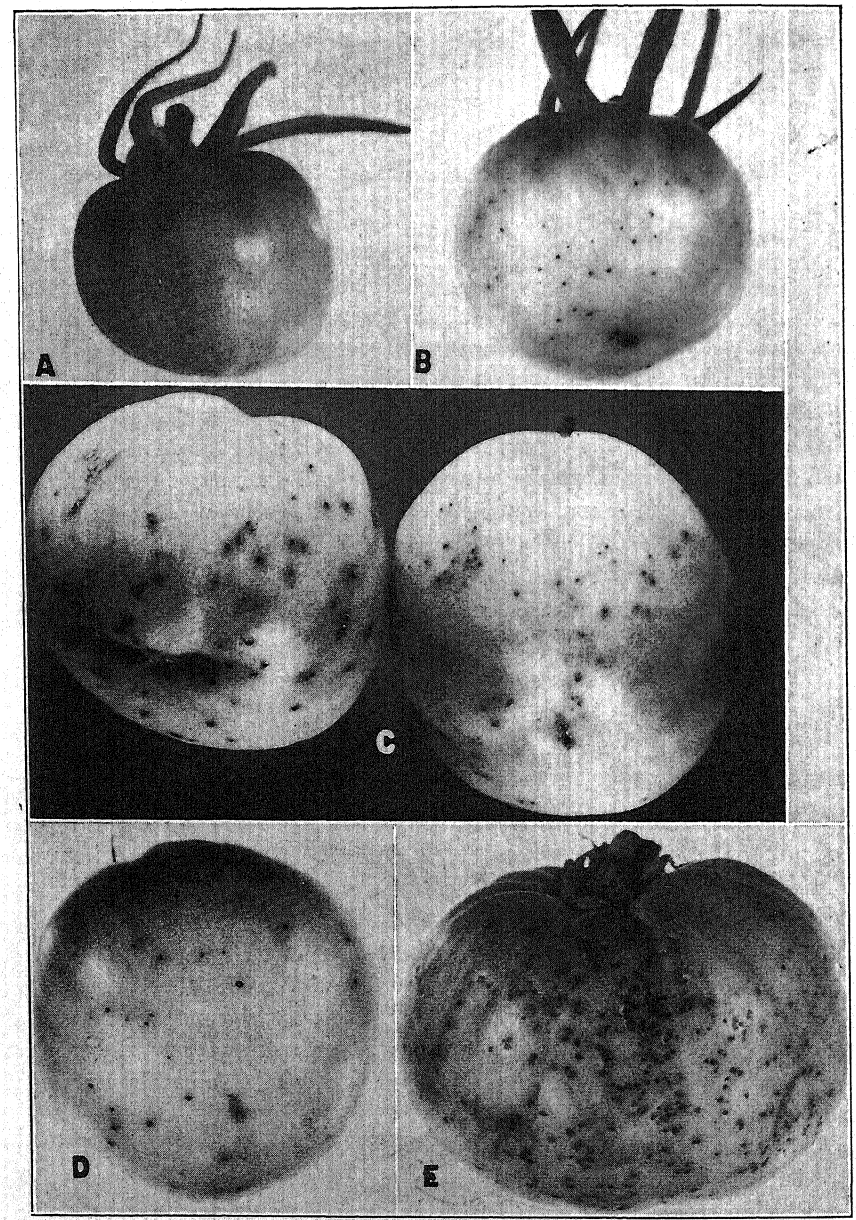


FIG. 1. Bacterial speck on tomato fruits. A. and B. Early stages. A, 7 days and B, 10 days after inoculation. C. Fruits in early stages of ripening showing darker green areas around the spots 15 days after inoculation. D and E, 21 days after inoculation. D. Scattering spots. E. Crowded spots.

D and E.) The spots are very slightly raised, flat and dark when first observable. (Fig. 1, A.) At this early stage, as well as when fully developed, they are round and flat with a clear-cut margin. As the fruit whitens before the pink stage, the tissue immediately surrounding the spots remains green but without clearly defined margins. (Fig. 1, C.)

The size of the spots depends on the age of the fruit when inoculated, those on very young fruit attaining the largest size. The epidermis remains unbroken. Lesions are, at first, very superficial. The epidermis is raised and separated from the underlying tissues by a dense mass of bacteria. Later, there is somewhat deeper penetration, with an attempt to cork off the intruder. The spot never penetrates so deeply as either bacterial spot or bacterial canker.

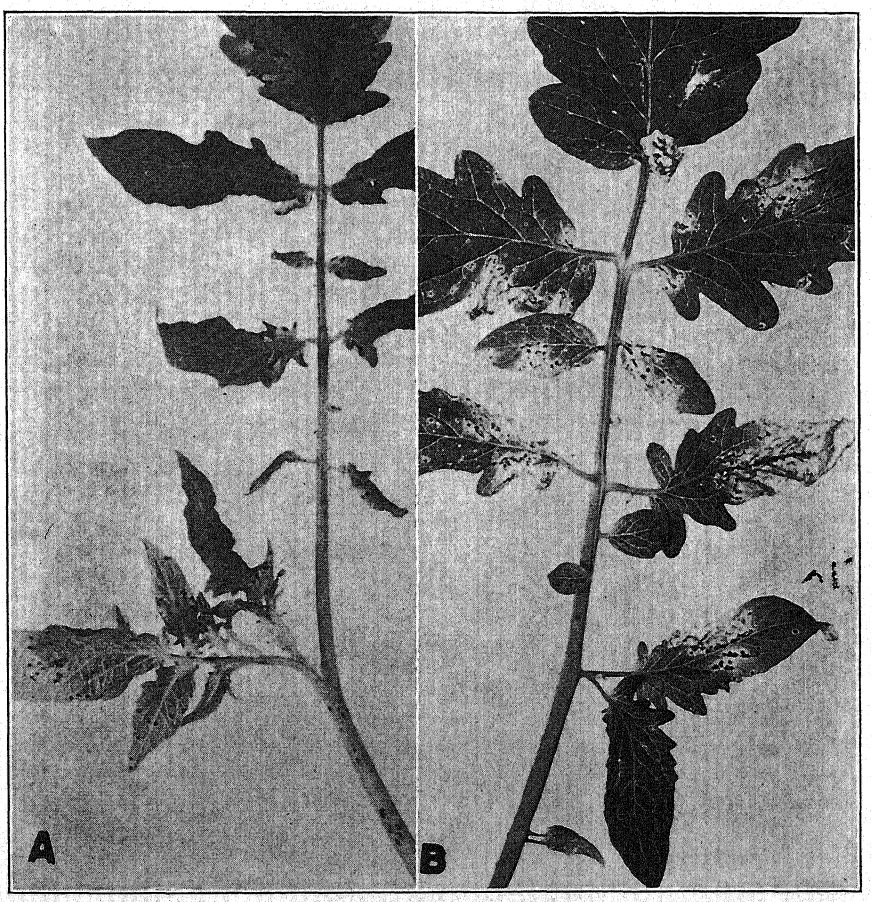


FIG. 2. Spotting on tomato stem and leaves inoculated with *Bact. punctulans*. A. 6 days after inoculation. B. 1 month after inoculation.

Lesions on the leaves are round, dark brown or black, without halo of any sort in early stages, and do not attain any larger size than the fruit spots. (Fig. 2, A.) They extend the entire thickness of the leaf, showing more conspicuously on the lower, paler surface. When close together spots coalesce into large irregular blotches and the surrounding tissues become yellow. (Fig. 2, B.) The petiole and petiolules also may be attacked.

Infections have been produced on stems, petioles, peduncles, pedicels, and sepals. In all these positions the spots tend to be oval rather than round, elongated with the long axis of the organ concerned. The peduncle

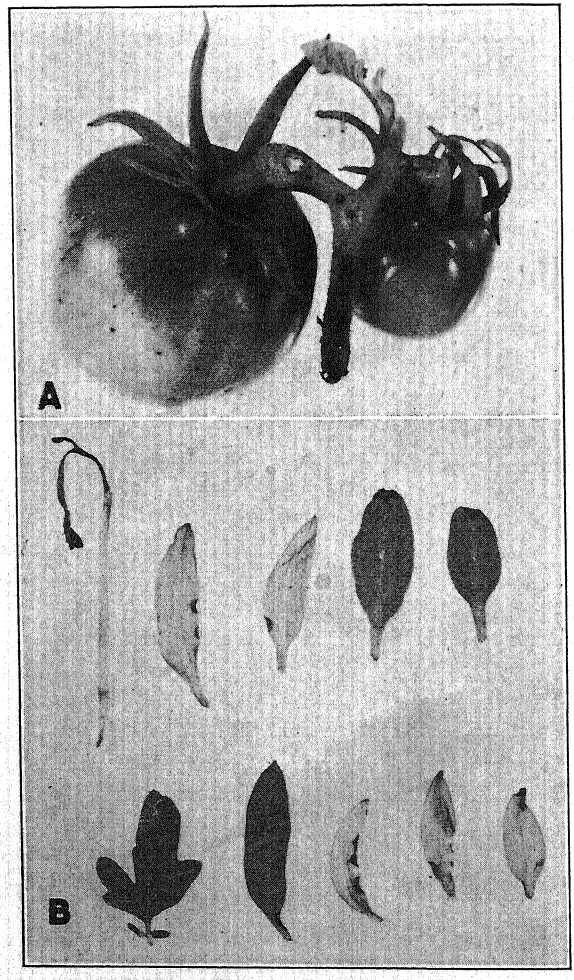


FIG. 3. A. Lesions on peduncle. B. Lesions on seedling leaves and on a seedling stem from inoculated seed.

seems especially susceptible. Here, lesions often coalesce to form large spots. (Fig. 3, A.)

INOCULATIONS

Inoculations on the fruit were made in two ways: 1, by swabbing lightly with a wad of cotton dipped in a water suspension of the organism; 2, by thrashing the fruit lightly with a leaf, simulating storm injury, then spraying with the water suspension. As with other bacterial spots of the tomato fruit, infection takes place through wounded hairs, there being no stomata present. Signs of infection are first observable after 5 days, as minute light brown points. These enlarge slowly. When very virulent strains were used on vigorous, young fruit, the minute infections in early stages oozed bacteria in such quantities as to show as white cirri. Only young fruits are susceptible.

Leaves and stems were inoculated by thrashing with a leaf followed by spraying with the inoculum. It appears that here, as well as on the fruits, the parasite enters through wounded hairs, for many more infections are obtained when the hairs are injured by thrashing than when the uninjured leaves and stems are sprayed and kept moist in inoculation chambers. Infections on seedlings have been obtained by planting clean seed in inoculated soil and also by planting inoculated seed in clean soil. Dark lesions appear on the cotyledons soon after they emerge, usually on the margins, but do not attain more than 1 mm. in diameter. Lesions also occur on the young stem and, in such cases, the whole top becomes involved, bends over, and blackens. (Fig. 3, B.)

Attempts were made to infect other cultivated plants. Seedling and well-grown eggplants, bell peppers and pimentos were inoculated by spraying the leaves and by swabbing young fruit, without obtaining any infections. Inoculations on bean, cucumber, and squash leaves also failed to produce infections.

THE ORGANISM

Morphology and Staining Reactions: Bact. punctulans is a rod varying from 1.3 to 2.5 μ long by 0.6 μ wide, measured on preparations from 24-hour pH 7.0 beef agar slants, occurring mostly as single rods or pairs. Long chains and filaments are also formed. The rods are motile by means of a tuft of several (as many as 7 were counted), polar flagella. Slender capsules were demonstrated by Ribbert's dahlia stain. No spores are formed. The organism is Gram-negative, not acid-fast, and stains readily with the usual bacterial stains.

CULTURAL CHARACTERS

Beef-infusion agar pH 7.0: Colonies on beef-agar plates are visible within 24 hours, and by the second day are 2 mm. wide, white, round, translucent,

and finely cross-hatched by oblique light. Later, they develop a more opaque white center and may reach a diameter of 9 mm., while the substratum becomes more or less green.[1] Occasional rough colonies appear on isolation plates. These are irregular in outline with a contoured or roughened surface and coarse cross-hatching. Platings from strains that have been on culture media for some time show more variations in colony type than platings direct from the plant. On slants the growth is moderate, spreading, flat, transparent, glistening, with slightly undulant margin. The medium becomes green.

Thaxter's potato agar: On this medium growth is abundant but not fluid; smooth, white, and opaque.

Beef-infusion broth pH 7.0: Clouding occurs within 24 hours and becomes heavy with membranous pellicle. In undisturbed cultures the pellicle is weighed down by a mass of white growth accumulating in the center. Green fluorescence develops after two or three days.

Potato cylinders: Growth is very moderate, inconspicuous, and transient. The substratum is slightly browned.

Synthetic media: Uschinsky's solution becomes heavily clouded with a membranous pellicle like that in beef broth. The medium quickly takes on a beautiful blue-green fluorescence. In Fermi's solution there are less clouding, a fluorescence more blue than green, and a heavy pellicle. Extremely weak but definite growth occurs in Cohn's solution.

PHYSIOLOGY

Liquefaction of gelatin. Liquefaction of beef gelatin at 18°–22° C. is napiform, beginning within 24 hours. Fifteen to 20 days are required for complete liquefaction. Abundant flocculent precipitate accumulates in the funnel. The medium becomes green.

Reactions in milk. Litmus milk begins to blue within 24 hours. Separation begins at the same time and in 5 days the semisolid curd occupies three-fourths to one-half of the culture. There is no banding. Reduction begins on the third to the fifth day and is complete within 2 weeks. The cream rim is reddened. The curd becomes firm in about 2 weeks. No peptonization occurs.

Hydrolysis of starch. Starch is not digested. Growth on potato cylinders is restricted and transient. On starch agar plates, flooded with iodine solution, after 7 days' growth, there is a slightly paler area immediately surrounding the growth bu no clear zone.

[1] Some strains are very strongly green fluorescent, others very weakly so, and some show no fluorescence in any medium. The nonfluorescent strains show also weaker virulence and are less vigorous in their reactions in culture media, especially litmus milk and gelatin.

Reduction of nitrates. There is strong nitrate reduction. Tests were made with the α-napthyl-amine-sulphanilic acid method, in cultures grown 10 days in nitrate beef broth.

Fermentation of sugars. Synthetic agar containing brom-cresol purple and 1 per cent of the following carbonhydrates was used: sucrose, dextrose, maltose, lactose, glycerine, and mannit. Slants were streaked from young broth cultures. No acid was formed from lactose or maltose. Acid reaction was evident in 24 hours from sucrose, dextrose, and mannit, and in 4 days from glycerine. After 6 days the agar was acid throughout with dextrose and in 10 days with sucrose, mannit, and glycerine. The same media, but without agar, were used in fermentation tubes. No gas was produced from any of the carbohydrates used. There was no growth evident in the closed end of any of the tubes, although the medium became acid in dextrose.

Relation to free oxygen. The organism is aerobic.

Optimum pH for growth. In a series in beef broth ranging from pH 5.0 to pH 9.0 clouding appeared within 24 hours in 6.0 to 8.7, best in 6.6 to 7.4, weak in 6.0 and in 7.6, and very weak in 8.3 and 8.7. After 48 hours the 5.4 showed growth. At the end of a week clouding was heavy in all from pH 5.4 (pH 7.0 at this date) to 7.6 (pH 8.1 at this date), moderate in 8.3, and weak in 8.7. All were green fluorescent from pH 7.4 to 8.7, most strongly so in 7.4 and 7.6.

Other physiological reactions. Bacterium punctulans does not form hydrogen sulphide or indol. Ammonia is formed slowly. Strips of filter paper dipped in Nessler's solution and suspended in 10-day-old beef-broth cultures did not show the ammonia reaction, while in 20-day cultures the reaction was good.

Temperature relations. The thermal death point is 51° C. The optimum temperature for growth is 23° to 25°. Growth is weak at 30°. At 35° and 37° beef-broth cultures not only did not grow but failed to cloud the medium when returned to room temperatures after 6 days at these temperatures. Slow growth with eventually good clouding occurs at 15° C. Clouding is visible in beef broth at 3° C. within 6 days.

Resistance to desiccation. The organism is very sensitive to drying. Preparations on cover glasses from 24-hour pH 7.0 beef bouillon, as well as from 3-day bouillon cultures, failed to give growth when dropped into bouillon after 48 hours' drying. One strain survived 9 days on three of ten covers placed in bouillon after that period, but required 8 days to show clouding.

BRIEF CHARACTERIZATION

Bacterium punctulans is a rod-shape bacterium, occurring singly, in pairs, and in chains, measuring 1.3–2.5 μ × 0.6 μ, motile by means of a tuft of polar flagella. It is Gram-negative and not acid-fast; forms thin, white

colonies with green fluorescence on beef agar; clouds bouillon heavily with a membranous pellicle, heavy in the center; no diastatic action; liquefies gelatin rapidly; forms acid without gas from sucrose, dextrose, glycerine, and mannit but not from lactose or maltose; reduces nitrates; blues, coagulates, and reduces litmus milk; is aerobic; produces ammonia but not hydrogen sulphide or indol; grows best in pH 6.6 to 7.4, is very sensitive to drying; optimum temperature 23°–25° C. Pathogenic to tomatoes.

SUMMARY

A disease of tomatoes designated as speck, heretofore confused with bacterial spot (*Bacterium vesicatorium*), is described. Speck has been found in Florida and in Maryland. It causes small, round, dark, superficial spots on tomato fruits. Stems and leaves also are readily infected. The bacteria enter through wounded hairs. The causal bacterium belongs to the green fluorescent group and is designated as **Bact. punctulans,** n. sp. Cultural and physiological characters of the organism are given.

BUREAU OF PLANT INDUSTRY,
U. S. DEPT. OF AGRICULTURE,
WASHINGTON, D. C.

REACTION OF MAIZE SEEDLINGS TO GIBBERELLA SAUBINETII[1]

H. K. HAYES, I. J. JOHNSON, AND E. C. STAKMAN[2]

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The use of resistant varieties in controlling plant diseases caused by fungi is dependent upon the extent to which it is possible to obtain resistant varieties satisfactory also in other characteristics. A number of root-rotting organisms attack corn, and among these *Gibberella saubinetii* (Mont.) Sacc. has received considerable attention. Cooperative studies carried on by the Divisions of Plant Genetics and Plant Pathology have had as their aim the isolation of resistant selfed lines of corn and their recombination in crosses, to ascertain whether lines could be isolated that would remain resistant and whether such resistance was of practical importance in corn improvement.

The present report consists, for the most part, of a study of later generations from crosses reported previously by McIndoe.[3]

PREVIOUS STUDIES

A review of the previous investigations having a more or less direct bearing on the problem was made by McIndoe in 1931.[3] In brief, Dickson and coworkers[4] have shown that the extent of seedling blight caused by Gibberella is dependent largely upon soil temperature. After isolating resistant and susceptible lines of corn they learned also that susceptible strains were severely injured at temperatures below 24° C., while resistant strains were attacked below temperatures of 16° C. This led to the method of studying resistance and susceptibility of seedlings grown under controlled conditions in the greenhouse.

Dickson thought that the host plant was least susceptible when grown at temperatures most favorable to its development, *i.e.*, under conditions of normal metabolism; at temperatures above 24° C., the host plant would

[1] Cooperative studies between the Division of Agronomy and Plant Genetics and the Division of Plant Pathology and Botany. Paper No. 1148 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Chief and Assistant Professor, Division of Agronomy and Plant Genetics, respectively, and Head, Section of Plant Pathology, Division of Plant Pathology and Botany.

[3] McIndoe, K. G. The inheritance of the reaction of maize to *Gibberella saubinetii*. Phytopath. 21: 615–639. 1931.

[4] Dickson, J. G. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetii*. Jour. Agr. Res. 23: 837–870. 1923.

———. The relation of plant physiology and chemistry to the study of disease resistance in plants. Jour. Amer. Soc. Agron. 17: 676–695. 1925.

succeed in throwing off attacks of the pathogen, but, if susceptible lines were grown at lower temperatures, the abnormal metabolism permitted the development of the pathogen. Peterson, however, concluded that the resistance of corn seedlings to attacks of root-rotting organisms is dependent upon the ability of the seedlings to develop a functioning nodal root system before the mesocotyl is completely rotted.

Dickson and Holbert[5] concluded that first-generation crosses between resistant and susceptible strains of corn were susceptible at all temperatures, *i.e.*, that there is complete dominance of susceptibility.

McIndoe used the most resistant and susceptible lines isolated previously in the corn-breeding studies carried on at the Minnesota station. He studied also the reaction of a large number of F_1 crosses in comparison with that of the parental lines when grown under comparable controlled temperature conditions in the greenhouse. These crosses consisted of the F_1 combinations between long-time selfed lines, which were being used in learning the more desirable combinations. Conflicting results were obtained in the F_1 generations. Certain F_1 crosses were highly resistant, being more resistant than either parent; others were more susceptible than either parent, although, in general, there was little or no relationship between the reaction of the parents and of the F_1 crosses. It would seem from these results that the causes of resistance are very complex. McIndoe studied the reaction of F_3 lines selected at random and included in the study crosses between resistant × susceptible, susceptible × intermediate, and susceptible × susceptible parents. There appeared to be a somewhat higher degree of resistance in the F_3 lines from crosses, one of whose parents was classed as resistant, than in the F_3 lines from crosses between parents both classed as susceptible, although the type of segregation appeared relatively complex.

EXPERIMENTAL MATERIAL AND METHODS

The same strain of *Gibberella saubinetii* studied previously by McIndoe has been used also in these studies. As reported by McIndoe, the soil temperature rarely varied more than 2° C. about a mean temperature of 15° C.

Seed from the cultures to be tested was divided into 2 parts, one of which was treated with Semesan solution and planted in the controlled-temperature greenhouses to determine normal viability of the seed. The other part was inoculated with a pure culture of *Gibberella saubinetii* grown previously for about 10 days in a moist mixture of wheat and oat grains. The inoculum was prepared by adding tap water to the mixture, straining through cheesecloth, and standardizing by dilution, so that the concentration of

[5] Dickson, J. G., and J. R. Holbert. The influence of temperature upon the metabolism and expression of disease resistance in selfed lines of corn. Jour. Amer. Soc. Agron. 18: 314–322. 1926.

spores was relatively uniform, the standard of comparison being 30–50 spores under the low-power field of the microscope.

The corn grains were planted in sand at uniform depth and the number of healthy plants was used as a criterion of the degree of resistance of the row. A correction was made on the basis of the germination obtained from the seed treated with Semesan Jr. in order to determine the extent of injury caused by the pure culture of Gibberella used in the studies.

The reliability of the method of study was determined by growing inoculated seed from each ear in 4 replications in 1930–31 and 3 in 1931–32, each test consisting of seeds planted in a single row in the greenhouse bench, the rows in each replicate series being randomized.

The host material consisted chiefly of later generations, F_4 and F_5, from the crosses reported by McIndoe. The studies were primarily of two sorts, correlation between reaction of rows in the different replications grown from seed of the same ears and interannual correlations to learn the extent to which manner of reaction was inherited under the conditions of the experiment.

The F_3 lines used by McIndoe consisted of F_3 progeny of random selections of F_2 selfed plants of the different crosses. F_4 progeny were grown and several ears were selfed in each line. The interannual F_3 to F_5 correlation coefficients reported consist of the relation between the seedling reaction of the progeny of the parent ear and the seedling reaction of the progeny of selfed ears of desirable plants of the following generation.

EXPERIMENTAL RESULTS

Because of the nature of the results it appears unnecessary to present them in great detail. The correlations between the reaction of replicates in 1930–31 are given in table 1. Crosses 43×46 and 43×47 are between resistant and susceptible lines, respectively, 49×50 between susceptible and intermediate parents, and 64×66 is the reciprocal type of cross, while both parents in the cross 58×60 were classed as susceptible.

The 5 crosses were studied separately, correlation coefficients being computed between the reaction of material grown in different replications.

All possible relationships were studied, *i.e.*, between replications 1 and 2, 1 and 3, 1 and 4, 2 and 3, 2 and 4, and between 3 and 4. All calculated coefficients were more than 3 times their probable errors, 13 of the 30 being over .5 and 17 less than .5. Considering the fact that only 10 seedlings were grown in each row, the extent of the relationship between different replicates seems rather satisfactory. When the average reaction between replications 1 and 2 was correlated with the results of the average of 3 and 4 all correlations were over .5, the smallest being $.5804 \pm .0437$ and the largest $.8425 \pm .0416$. These results appear rather satisfactory and in-

TABLE 1.—*Correlations between the reaction of replicates of F_4 progeny of crosses when inoculated with Gibberella saubinetii and grown under controlled temperatures. 1930–31*

Cross	Replicates 1 and 2	Replicates 1 and 3	Replicates 1 and 4
43 × 46	.6583 ± .0381	.5264 ± .0485	.4272 ± .0549
43 × 47	.2718 ± .0609	.3268 ± .0588	.4183 ± .0543
49 × 50	.5475 ± .0661	.5364 ± .0673	.6388 ± .0559
64 × 66	.4273 ± .1082	.3965 ± .1114	.6762 ± .0718
58 × 60	.8006 ± .0515	.7753 ± .0574	.6804 ± .0773

Cross	Replicates 2 and 3	Replicates 2 and 4	Replicates 3 and 4	(Av. 1 and 2) and (Av. 3 and 4)
43 × 46	.4424 ± .0540	.4085 ± .0559	.4427 ± .0539	.5812 ± .0445
43 × 47	.3844 ± .0561	.3567 ± .0574	.3822 ± .0562	.5804 ± .0437
49 × 50	.4576 ± .0746	.4813 ± .0726	.6841 ± .0503	.6675 ± .0544
64 × 66	.3444 ± .1166	.7892 ± .0499	.8720 ± .0317	.6641 ± .0740
58 × 60	.4407 ± .1158	.7693 ± .0588	.3603 ± .1252	.8425 ± .0416

dicate that the method of studying a short row grown from seed immersed in a water suspension of spores of *Gibberella saubinetii* is a fairly accurate means of determining the manner of reaction of seedling progeny of individual selfed ears.

Similar studies were made in the winter of 1931–32, with F_5 seedlings of the same crosses. Three replicates were used in this study and levels of significance determined by the use of Fisher's tables. In general, somewhat lower correlations were obtained than in 1930–31, although the calculated coefficients were for the most part significant. The extent of correlation ranged from +.270 to +.803. In the light of previous years' study it seems reasonable to conclude that the average of all three replications would show rather satisfactorily which of the F_5 lines were resistant and which susceptible.

TABLE 2.—*Correlations between the reaction of replicates of F_5 progeny of crosses when inoculated with Gibberella saubinetii and grown under controlled temperatures*

Crosses	Replicates 1 and 2	Replicates 1 and 3	Replicates 2 and 3	N	Approximate level of significance P = .01	P = .05
43 × 46	.430	.567	.397	83	.283	
43 × 47	.478	.803	.516	30	.449	
49 × 50, 58 × 60, and 64 × 66	.270	.361	.393	41	.393	.304

To be of value as a means of isolating resistant lines there should be a substantial relation between the reaction in different seasons: some lines should remain constantly resistant, while others should be consistently susceptible. Interannual correlation coefficients for the reaction between F_3 and F_4 as well as F_4 and F_5 are given in table 3. Studies are given also for selfed lines of Crosby and Golden Bantam.

TABLE 3.—*Interannual correlations of reaction of maize seedlings to seedling blight*

Nature of correlation	Years selfed	N	r	Approximate level of significance P = .01
43 × 47 (R × S)	F_3 and F_4	121	.095	.2540
43 × 46 (R × S)	F_3 and F_4	111	.074	.2540
49 × 50 (S × I)	F_3 and F_4	53	.092	.3541
58 × 60 (S × S)	F_3 and F_4	23	.312	.5368
64 × 66 (I × S)	F_3 and F_4	29	.133	.4487
43 × 47 (R × S)	F_4 and F_5	42	.055	.3932
43 × 46 (R × S)	F_4 and F_5	85	.205	.2830
(49 × 50), (58 × 60), and (64 × 66)	F_4 and F_5	43	.060	.3932
Crosby	3 and 4 yrs. selfed	40	.536	.3932
Golden Bantam	3 and 4 yrs. "	60	.280	.3248

In this study the reaction of F_3 seedlings of a particular selfed ear was correlated with the reaction of F_4 seedlings of selfed ears of the same progeny line. Similar methods were used to correlate F_4 and F_5. In the progeny of the various types of crosses none of the correlations are of significance. This leads to the conclusion that reaction of seedlings under controlled temperatures to attacks of *Gibberella saubinetii* cannot be used as a means of isolating resistant lines unless conditions are controlled to a greater extent than in the experiments reported here.

During 1930–32 a study was made of the interannual correlation between the reaction of 3-year selfed lines with that of the 4th-year selfed, using 40 lines of Crosby and 60 of the Golden Bantam. In this case both correlations were positive and that for the 40 Crosby lines was .536, with a level of significance for a P value of .01 of .3932. Most of these sweet-corn lines appear very uniform under normal field conditions, and the extent of homozygosis may explain why they gave a sensible relation between the reaction of 3rd- and 4th-generation selfed lines.

In the plant-breeding studies with corn, yielding ability of selfed lines is obtained by husking the ears of 10 plants and estimating the yield on a scale of 1 to 10. A considerable number of selfed lines of Golden Bantam

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and Crosby sweet corn have been treated in this manner and their yield index correlated with reaction to *Gibberella saubinetii*. The results of this study are given in table 4, together with correlation between length of ear of the selfed plant and its seedling-blight reaction.

TABLE 4.—*Correlations between seedling reaction to Gibberella saubinetii and indices of plant vigor*

Characters correlated		N	r	Approximate level of significance P = .01
Gibberella reaction and ear length:				
Golden Bantam	5 years selfed	52	−.149	.322
" "	4 " "	100	.084	.254
Crosby	4 " "	49	.113	.358
Gibberella reaction and yield index of the parental line:				
Golden Bantam	4 years selfed	60	.285	.326
" "	3 " "	205	.048	.254
Crosby	4 " "	46	.046	.372
"	4 " "	50	.061	.358
"	3 " "	252	.0001	.254
Gibberella reaction and yield index of its progeny:				
Golden Bantam	3 years selfed	43	.029	.393
Crosby	3 " "	62	.048	.325

Of the 10 correlations given in table 4 only one between Gibberella reaction and yield index in 60 4-year selfed lines of Golden Bantam was of possible significance.

SUMMARY AND CONCLUSIONS

The manner of reaction of corn seedlings to attacks of *Gibberella saubinetii* was determined by inoculating seed with a pure culture of the organism and planting the seed in sand in a greenhouse where the soil temperatures were controlled within a range of approximately 2° C., an average soil temperature of 15° C. being maintained.

There was a sensible relation between the reaction of replicates when seedling progeny from the same ears were studied, indicating that the method was sufficiently accurate to be used as a means of isolating lines that might be consistently resistant or consistently susceptible.

Interannual correlations, expressing the extent to which there was an inheritance of manner of reaction to *Gibberella saubinetii*, were for the most part so small that it seems safe to conclude that the method is not reliable as a means of isolating lines that may be expected to behave in a consistent

manner. In other words, the progeny of individual ears were relatively constant in the extent of seedling blight, although inheritance was not apparently the main cause of uniformity of reaction in different replicates. It seems safe to conclude, therefore, that the conditions under which an ear develops determines in some way the manner of reaction of the progeny.

The results of these studies have made it apparent that a study of seedling blight in the manner here outlined is of little or no value in a corn-breeding program. This has led to the treatment of a part of the seed of each selfed ear with Semesan Jr. and the comparison of viability under field conditions with the progeny of nontreated seed. In future studies it is planned to inoculate a part of the seed of each selfed ear with Gibberella, and another part with Penicillium before planting in the field and compare the results with seed treated with Semesan Jr.

No significant relationship was obtained between reaction to Gibberella and indices of plant vigor.

INFLUENCE OF SPRAY APPLICATIONS ON AIR TEMPERATURES SURROUNDING SPRAYED POTATO PLANTS

H. R. Rosen

(Accepted for publication, Jan. 9, 1933)

For a number of years the writer has been attempting to determine the influence of such biologic and ecologic factors on the origin of tip and margin burn of Irish potato leaves as the relationship of leaf hoppers and flea beetles to such injury and the influence of light intensity, wind velocity, relative humidity, precipitation, amount of soil moisture, and air and soil temperatures.

Since two different types of etiologic agents have been postulated in the literature of potato tip and margin burn, one associated with the leaf hopper, *Empoasca fabae,* and the other with dry, hot weather, it seemed desirable, in view of the extreme prevalence of such injuries in Arkansas, to determine which of these factors was commonly involved in this State and to determine the efficacy of various control measures. This paper records precipitous drops in air temperature noted in conjunction with these studies.

Numerous investigators have found that Bordeaux mixture applied to Irish potatoes acted as a preventive for tip and margin burning, particularly against the type induced by leaf hoppers. Accordingly, this spray material among others has been used by the writer for several years with excellent results in controlling tip and margin burning in the northern, hilly part of Arkansas, and with little or no result up to the present in the southern, coastal plain part of the State.[1]

Indeed, any control of hopper injury obtained at Hope has been more than offset by the amount of spray injury encountered there and in marked contrast with that at the cooler, northern station at Fayetteville, where no such injury with Bordeaux sprays has occurred. In fact, marginal leaf burning has been so common at Hope in plots sprayed with Bordeaux mixture 4–4–50 and similar formulas, with symptoms very comparable to leafhopper injury, that, so far as concerns leaf injury, it has been difficult to distinguish sprayed from nonsprayed plants. These leaf injuries associated with spraying were absent or very slight in the first half of the potato-growing seasons and became more pronounced as the season advanced.

What is the reason for this discrepancy? An attempt to answer this question included a consideration of various injurious effects that might derive from Bordeaux mixture under certain climatic conditions. The

[1] The results here recorded were obtained at the Fruit and Truck Branch Experiment Station, Hope, Arkansas.

answer is not certain, but, in the course of analyzing the thermographic records of soil and air temperatures prevalent around sprayed and nonsprayed plants, the charts in almost every instance revealed very interesting drops in air temperatures occurring immediately after the application of the sprays, particularly during the latter half of the growing season. The thermographic records of nonsprayed plants representing the same intervals of time as the records of the sprayed ones show no such drops (Fig. 1).

Out of 23 sprayings conducted during a 3-year period, only 2 show no positive indications of immediate drops in temperature following the application. Table 1 records the changes that occurred in 2 of these years and presents the temperature, wind velocity, precipitation, and relative humidity prevalent on the respective dates of spray applications.

It will be observed in table 1 that no reductions in temperature were recorded in 2 instances and that all the other sprayings were accompanied

TABLE 1.—*Weather conditions at the time of spray applications and the drops in temperatures attending the spraying of potato plants*

Date and hour sprayed	Temperature at time of spraying	Wind velocity at 6 p. m.	Precipitation during 24 hours previous	Relative humidity at 6 p. m.	Drop in temperature
1930	°C.	*m. p. hr.*	*in.*	*per cent*	°C.
April 22, 4 p. m.	32.0	1.6	0	76	5.0
May 7, 4 p. m.	28.5	5.4	0.83	83	6.0
May 14, 8 a. m.	22.0	2.3	0	61	0
May 21, 5 p. m.	28.0	1.2	0	76	4.0
May 28, 1: 30 p. m.	30.5	1.2	0	83	2.5
June 4, 4 p. m.	32.5	2.3	0	63	4.5
June 11, 5: 45 p. m.	29.0	2.4	0	53	3.0
June 18, 4: 15 p. m.	37.0	1.5	0	60	7.0
1931					
April 23, 11 a. m.	20.0	1.7	0.45	77	drop not clear (ink spot)
April 30, 3 p. m.	22.5	2.6	0	90	0 (rain immediately after)
May 7, 11 a. m.	21.0	3.1	0	64	4.0
May 14, 11 a. m.	27.0	2.2	0	50	5.0
May 21, 9: 30 a. m.	22.5	3.1	0	55	4.0
May 28, 8 a. m.	25.0	2.1	0	57	1.5
June 4, 2 p. m.	38.0	2.7	0	46	8.0
June 11, 8 a. m.	26.0	3.4	0.01	64	2.0
June 20, 10: 30 a. m.	34.0	2.7	0	71	5.5
June 25, 4: 30 p. m.	32.5	2.3	0	91	3.0

by a decided drop, varying in amount at different times. Unfortunately, the relative humidity and wind velocity were measured, not at the time of spraying but at 6 p. m. of the same day. Since these may be expected to vary considerably in any one day, the data on these 2 factors are not so satisfactory as they might otherwise be. It is to be noted that on May 14, when no drop was recorded, the spraying occurred at 8 a. m., when the air temperature (22.0° C.) was relatively low and on the other occasion, April 30, 1931, when rain fell immediately after the spray was applied. As may be expected, the greatest temperature drops occurred when the spraying was conducted near midday or in the afternoon, the largest drop being recorded at 2 p. m., June 4, 1931, when the relative humidity was very low and the temperature very high.

A clearer idea of the nature of these temperature drops may be gained by observing 2 air-temperature graphs of sprayed and nonsprayed plants (Fig. 1).

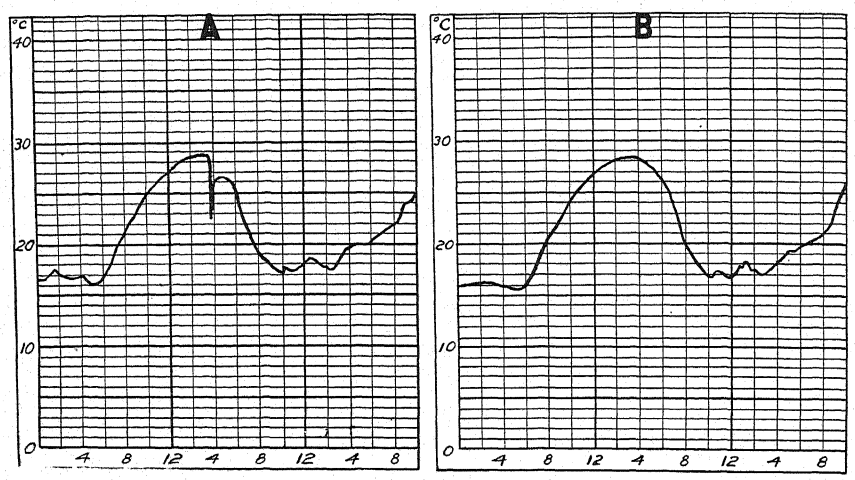


FIG. 1. A. Air temperatures existing around sprayed potato plants at Hope, Arkansas, from midnight, May 6, to midday May 8, 1930. Note sudden and precipitous drop in temperature shortly before 4 p. m. on May 7, when Bordeaux spray was applied. B. Air temperatures existing around nonsprayed potato plants at the same time and place as shown in A.

The temperature drop shown in figure 1, A, occurred, according to the records, shortly before 4 p. m. on May 7, 1930, immediately after the spray had been applied. The thermograph record shows a precipitous drop of 6.0° C. (12.6° F.) immediately followed by a perpendicular upward curve, coinciding almost exactly with the downward curve. The control plants (Fig. 1, B) showed no such abrupt change in temperature.

The thermograph instruments enclosed within housings comparable to those used by the United States Weather Bureau were placed at levels representing approximately the medium height of the potato plants, one between two sprayed rows and the other between two nonsprayed rows. The rows in each instance were 3 feet apart, and the temperature-sensitive portion of each instrument was, accordingly, about 18 inches from the center of each row. With the foliage extending about a foot outward from each side of the middles, the instruments were in excellent positions to record temperature changes occurring around the plants. However, considering the width of the instrument housing and its possible insulating effect, it is conceivable that greater temperature changes may have occurred immediately around the plants than were shown in the automatically recorded charts. This is particularly likely to be the case when the changes are of such short duration as shown in figure 1, A.

For many years it has been known that spraying various kinds of plants during the warm hours of the day may be attended by injury to foliage and fruit. The factors that are responsible for such injury are, however, obscure and, in many cases, unknown. Of course, the burning action of a drop of liquid, in functioning as a convex lens and centering the beams of light, is well known. Likewise, the intolerance of certain plants to some spray materials has been noted by numerous observers. It also is known that certain spray materials, such as Bordeaux mixture and lime sulphur, may cause foliage injury and fruit russeting or burning under some weather conditions and not under others. That Bordeaux mixture will often cause a russeting of apple fruit when applied in the early part of the growing season and produce little or no injury thereafter is well known. Why this is so is not clear.

As far as potato plants are concerned, it has been definitely established that a Bordeaux spray increases the rate of transpiration (1, 3, 4, 5, 6, 7, 8). What is responsible for the rapid and precipitous drops in temperature when this spray is applied, especially during the warm hours of the day? The answer probably is to be found in the evaporating power of the warm, dry air. The transformation of energy occasioned by such evaporation evidently results in a rapid lowering of temperature.

Thus, two different phenomena may attend the spraying of potato plants and probably other plants as well, one, a quick lowering of air temperatures around the sprayed parts and the other, an increase in the rate of transpiration. It has not been shown, however, that the two occur simultaneously. It is conceivable that other liquid sprays may bring forth similar responses. Investigators as a whole have not associated any injury with increased transpiration. On the contrary, they have found that, despite such increases, the potato foliage does not suffer, even during dry seasons, and the yield of tubers is often increased, even in years when the control

of late blight and other foliar disturbances are not involved (2, 9). What influence may sudden changes of air temperature have on potato plants? Can changes of such short duration as here shown have anything to do with the spray injury that has been noted at Hope, Arkansas, for 4 successive years?

Briefly summarizing, while effective control of tip and margin burning of potato leaves has been obtained by the use of Bordeaux sprays in the northern part of Arkansas, no satisfactory control with this material has been accomplished in the southern part during a 4-year period. In attempting to determine the cause of this discrepancy and to find an explanation for the spray injury, which that material has engendered in the southern portion of the State, a study has been made of various climatic factors existing around sprayed and unsprayed plants. It has been found that spraying in most instances is accompanied by sudden and precipitous drops in air temperature around sprayed plants, the drops varying from 1.5° to 8.0° C. (3.1 to 16.8° F.). What influence such abrupt temperature changes may exert on the health of the plant remains unknown. It is quite obvious, however, that in the warmer sections of the State, with prevailingly high temperatures during the latter half of the potato-growing season, temperature drops attending spraying would be much greater than in the cooler sections. Does this suggest that midsummer spraying of various kinds of plants in the warmer parts of America is attended by greater risks in so-called spray injury than in the cooler parts?

AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARK.

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RESISTANCE OF THE CURRENT SEASON'S SHOOTS OF PINUS MONTICOLA TO INFECTION BY CRONARTIUM RIBICOLA[1]

H. G. LACHMUND

(Accepted for publication November 3, 1932)

In studies of the blister rust (*Cronartium ribicola* Fischer) on western white pine, *Pinus monticola* Dougl., (3), the writer found that the cankers resulting from any year's sporidial infection were distributed in the following characteristic pattern on the different years' internodes exposed to the infection.

	Growth[2] *affected*	*Number of cankers*	
a	Growth of the season of infection	relatively few	
b	Growth 1 year older than *a*	greatest number	large majority
c	Growth 2 years older than *a*	considerable number	large majority
d	Growth 3 years older than *a*	relatively few	
e	Growth 4 years older than *a*	very few	
f	Growth 5 years older than *a*	exceptional	
g	Growth 6 years older than *a*	none	
h	Growth 7 years older than *a*	none	

Tabulation, totalling approximately 6,000 cankers, gave about 10 per cent of the cankers on *a* growth, 53 per cent on *b*, 31 per cent on *c*, 5 per cent on *d*, less than 1 per cent on *e*, a trace on *f*, and none on older growths.

Cronartium ribicola infects its aecial hosts through the needles (1) on which it enters through the stomata and grows down the vascular bundles to the bark. In *Pinus monticola* it generally undergoes an incubation period of 2 years' duration before becoming apparent in the bark. Part of this period is required for the rust to develop in the needle and grow down from the infection point to the bark. The length of time required for this in *P. monticola* is not known. In *P. strobus* (1, p. 455) it appears to range from 2 to as high as 10 or 12 months and, in general, to be a matter of over 6 months.

Obviously, then, other conditions being equal, the number of cankers that develop on any year's growth as the result of infection in a given

[1] The writer was assisted by J. L. Mielke, C. N. Partington, W. V. Benedict, T. S. Buchanan, J. W. Kimmey, A. A. McCready, and W. F. Cummins in the collection of the basic data of this paper.

[2] The term "growth" is used to designate the internodes of a given year.

season should depend primarily on the quantity of needles on that growth and the length of time they are held following infection. Spaulding (6) has used similar premises for a partial explanation of differences in the susceptibility to the rust of different species of white pine.

In all growing trees there is normally a multiplication of twigs and branches and a progressive increase in foliage production each year to provide for the increased nutrition requirements of the tree as it increases in size. Each succeeding season's crop of shoots, therefore, has normally a greater total length, bark area, and production of foliage than that of the preceding season. Thus, from the standpoint of quantity alone, the chances of infection should be greatest on *a* growth and progessively smaller, the older the growth. These differences are very greatly and rapidly accentuated by the dropping of the leaves.

In the Pacific Northwest[3] (3) the needles of any season on *Pinus monticola* are regularly held for at least 3 full growing seasons. Part of them drop at the end of their third season, and most of the remainder fall at the end of the fourth season. The small portion left over are generally cast at the end of the fifth season, although, exceptionally, one or two needle bundles may persist as much as 8 seasons.

Infection of the pines in this region as elsewhere usually occurs in late summer and early fall. Figure 1 represents diagrammatically the normal retention of the needles at this time and the distribution of the resultant cankers, as shown by the tabulations already referred to above, in the second season following that of infection, when the cankers normally appear.

The reason for the negligible representations of cankers on *e* and older growths is immediately apparent. There are scarcely any needles on such growth at the time of infection, and such needles as are present normally fall at the end of the season or only shortly following the occurrence of infection. The relatively small representation of cankers on *d* growth is explained by the fact that such growth has already lost part of its needles and loses most of those remaining upon it at the end of the infection season. The smaller representation on *c* growth than on *b* growth may be explained on a similar basis. *C* growth loses part of its needles at the end of the infection season and most of the remainder one year later, whereas *b* growth holds all of its needles for at least a full year following infection and the majority of them for 2 years. Thus part of the individual infections on the needles of *c* growth may be cast with the needles at the end of the season before the fungus reaches the bark, while most of the infections on the needles of *b* growth should have ample time to gain entrance.

[3] Pacific Northwest as used in this paper includes southern British Columbia, Washington, Oregon, Idaho, and western Montana.

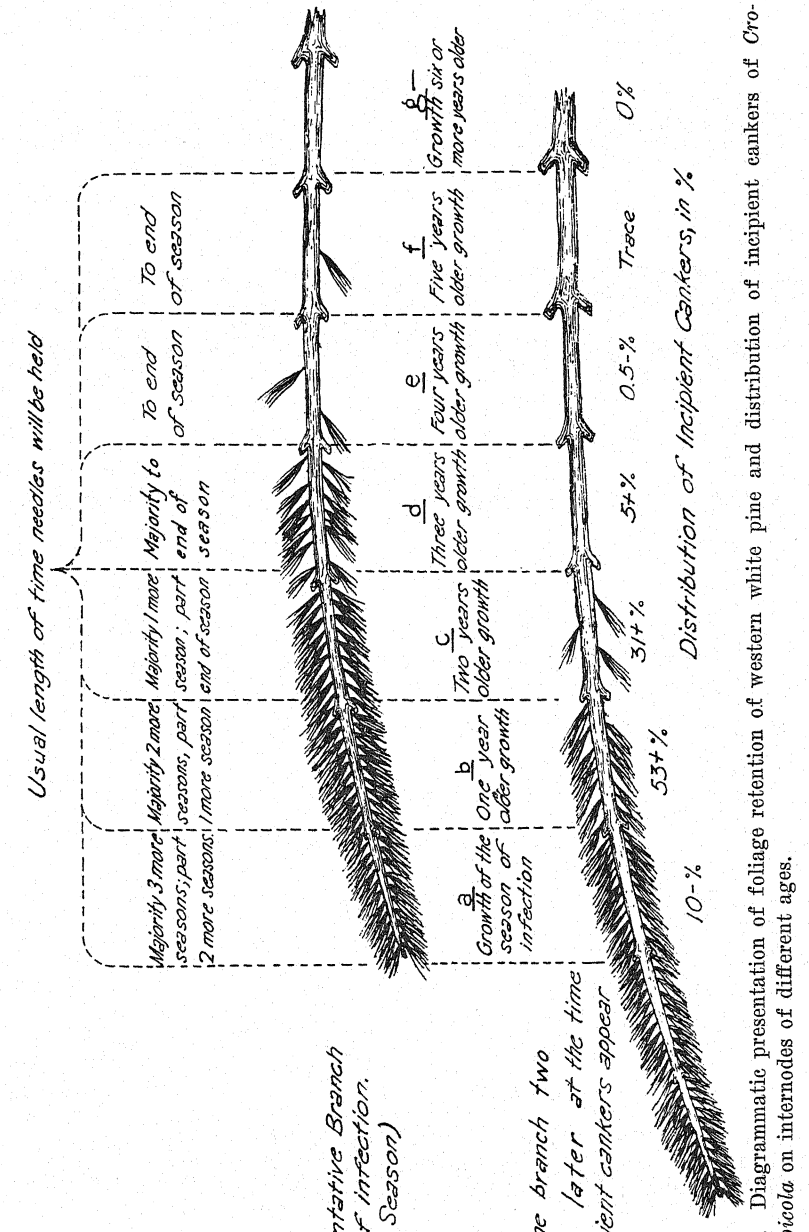


FIG. 1. Diagrammatic presentation of foliage retention of western white pine and distribution of incipient cankers of *Cronartium ribicola* on internodes of different ages.

On this basis *a* growth, which not only has a much greater number of needles but holds them a full year longer than *b* growth, should have a greater proportion of the cankers than *b* growth. Yet the percentage on *a* growth is on the average only a fifth of that on *b* growth and a third of that on *c* growth.

There appear to be no differences in the position of the needles on the tree, their arrangement on the branches or in the fascicles, their size at the time of infection, or in microclimatic conditions surrounding infection that could offset the superiority in the number and retention of needles on *a* growth to a degree sufficient to account for the inferiority in the number of cankers upon it as compared with *b* and *c* growths. Likewise, there is no evidence of any physiological barrier in the young bark tissues of *a* growth to the entrance of the mycelium from the needles. Indeed, comparing stems of equal size, the most vigorous canker growth is found on the youngest, thriftiest stems. Consequently, since especial vigor encourages the growth of the mycelium and since vigor is greater, the younger the shoot, it would not appear that there should be any physiological barrier in the bark of *a* growth that would prevent entrance of the mycelium, once the latter had reached the base of the needle. Direct infection of the bark may be eliminated as an explanation, for it is already established that this is not an important method of infection (1, p. 441; 5, pp. 26, 27), and if it were, the conditions for it would be primarily favorable on *a* growth (1, p. 441; 8).

The resistance indicated by the relative scarcity of cankers on *a* growth, therefore, evidently centers in the needles of this growth. Whether it arises as a physiological reaction of the young tissues or from differences in the behavior of the stomata, as in the case of the resistance of certain wheat varieties to stem rust (*Puccinia graminis tritici* Eriks. and Henn.) (2), or in some other way, is not known.

Regardless of where or how this resistance in *a* growth originates, it is clear that it is a function of age. That it persists through the period in which important infection may take place during the first season is also evident, for the number of cankers resulting from any season's infection on the growth that originated in the year of infection (*a* growth) has never been more than a third of the number resulting on the growth a year older (*b* growth).

The proportion of the cankers on *a* compared with the number on *b* has varied from as low as one-twentieth to one-third. The most probable explanation for this wide variation would appear to be that the resistance in *a* needles decreases rather rapidly following their formation, while the susceptibility in *b* needles remains relatively constant. It would necessarily follow that where the sporidial infection wave occurred early in the season

the proportion of cankers resulting on *a* would be relatively small, and where the infection occurred late in the season the proportion would be comparatively large.

Whether the resistance persists to any degree into the second season or later is not known. The solution of this problem will require artificial infection experiments.

The resistance in the current shoots of *Pinus monticola* is in direct contrast to the evidence in this connection for *P. strobus* L. On this species the needles of *a* growth appear to be quite as susceptible as those on *b* growth (9, p. 507) and *a* growth, as might be expected from its superiority in number and retention of needles, generally has the majority of the cankers (4, p. 479; 7, p. 38).

SUMMARY

Of the cankers resulting from any season's infection of blister rust on *Pinus monticola* relatively few are found on growth (internodes) formed the season of infection. Yet the needles, through which infection occurs on the pines (1), are not only more numerous but are held a year longer on this growth than on any older growth and for this reason should provide better opportunities for the subsequent entrance of the individual infections. Conditions governing the occurrence of infection, such as exposure to sporidia, humidity, etc., should be essentially as favorable on the season's needles as on the older needles. Observations indicate that conditions for the development of the fungus, once it is established in the bark, should be equally, if not more, favorable on the growth formed in the infection season than on growth one year older. But the number of the cankers that develop on the infection season's growth averages only a fifth of the number that develop on the one-year older growth and a third of the number that develop on growth 2 years older. The small proportion of cankers resulting on the growth of the season of infection is explained on the basis that its needles are resistant to infection. The resistance probably declines very rapidly during the first growing season and does not extend to any degree into the second growing season.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPT. AGR.,
PORTLAND, OREGON.

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PHYTOPATHOLOGICAL NOTE

The Spermatia of Corn Rust, Puccinia sorghi.[1]—Since the discovery by Craigie in 1927 of heterothallism in rusts and the function of rust spermogonia (pycnia) it has been known that spermatia (pycniospores) of one sex when brought to an infection of opposite sex can initiate there the sporophyte generation of the rust and lead to the development of fertile aecia. Several workers since then have attempted to learn just how the spermatia function, and Craigie has depicted the union of spermatia and haploid hyphae in *Puccinia helianthi* Schw. Much, however, still remains to be learned.

Puccinia sorghi Schw. has proved favorable material for such a study. It is a heterothallic, long-cycle, heteroecious rust with uredia and telia on corn and spermogonia and aecia on species of Oxalis.

Oxalis plants for this study were grown in 6-inch pots on an outdoor balcony and inoculated there with the rust. When the infections matured, the spermogonial exudate was transferred back and forth among the infections, the attempt being made to bring exudate to each infection from several others. In order to maintain a saturated atmosphere about the plants, a battery jar was lined with wet paper towelling and sprayed full of mist with an atomizer; the plants themselves were sprayed lightly; the inverted jar was set down over the plants; more wet towelling was wrapped around the joining of battery jar and pot-rim; and the whole was set in a partly shaded place. At short intervals infected material was removed from the plants; part of it was fixed, and the rest used for making freehand sections of the living material.

Figure 1, A, represents semidiagrammatically a cross section of the infected Oxalis leaf with intercellular mycelium and open spermogonia at *a* and *b*.

In figure 1, B and E, are sketches from the living material showing paraphyses with spermatia attached. In B is a brush of paraphyses with the outer line of the epidermis at *c*. A paraphysis may carry only 2 or 3 spermatia (Fig. 1, B, *a, b;* C, *c*) or may be crowded with them (Fig. 1, D, *a*). The spermatia may be applied closely to the paraphysis (Fig. 1, B, *b;* E, *b*) or may be at some distance and connected by a slender hypha (Fig. 1, C, *a;* E, *a*). It is not always possible to be sure how many of these

[1] Cooperative investigations between the California Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

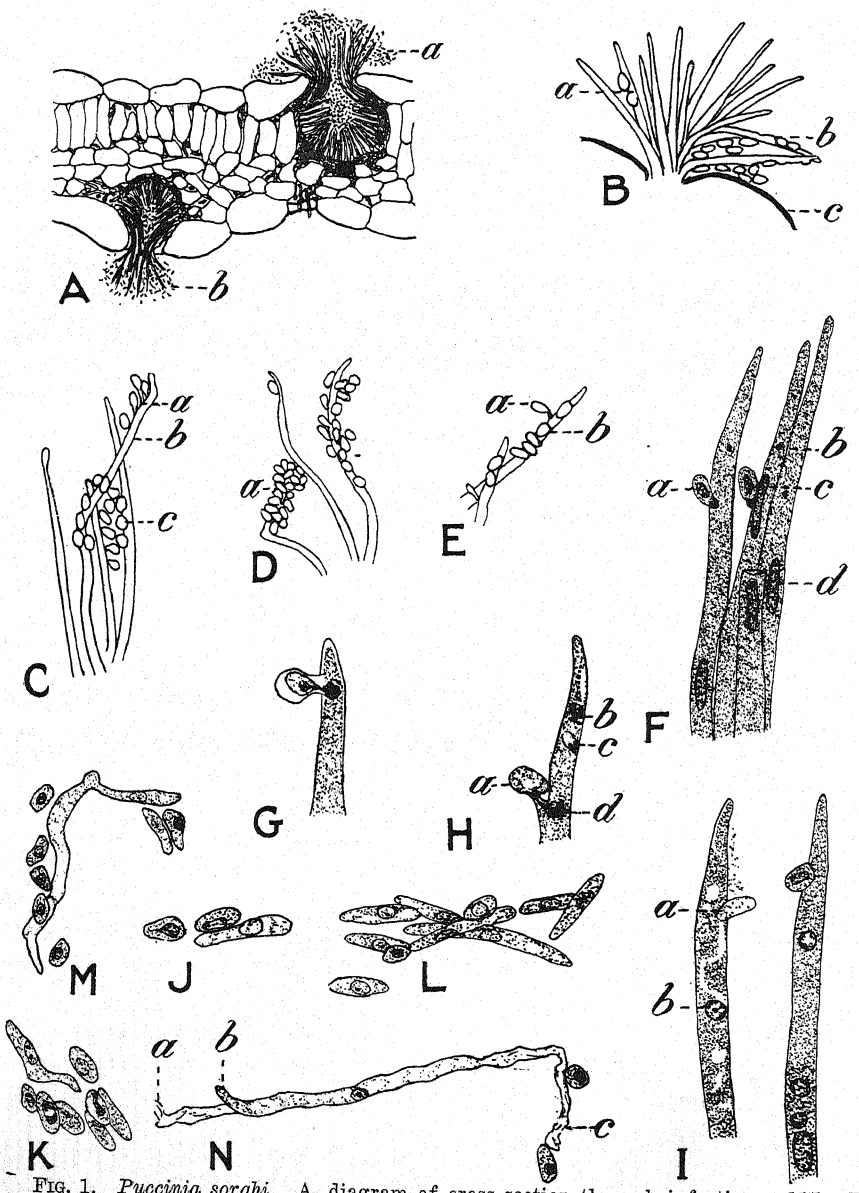


FIG. 1. *Puccinia sorghi.* A, diagram of cross section through infection, ×115. B to E, sketches from living material showing paraphyses with attached spermatia. Magnification not measured. F to I, stages of fusion of spermatium with paraphysis, ×1400. J to N, stages of germination of spermatia, ×1400.

spermatia are really attached to a paraphysis and how many are loosely adherent. As a precaution, before making the sketch, the cover glass was jogged and tapped repeatedly to free any loose spermatia. In some cases

the spermatia are certainly attached, for, by watching them under the microscope, spermatia like those at C, *a*, can be seen swinging back and forth when the cover glass is tapped.

In fixed and stained material the ordinary paraphysis is seen as a straight, rather stiff-looking cell, tapering at the tip. It contains a single nucleus, often somewhat elongated (Fig. 1, F, *d*) and dense cytoplasm sometimes bearing one or more dark-staining granules (Fig. 1, F, *b*).

A spermogonium forms new paraphyses as it grows older. When a plant has been in a saturated atmosphere for several hours, a newly emerging paraphysis may take on a different character. It grows more like an internal hypha in the saturated air of the intercellular spaces of the leaf. It is usually slender (Fig. 1, C, *b*), does not taper at the tip, follows an irregular course, and may branch. So far as noted, it is nonseptate.

Over 95 per cent of the spermatia attached to paraphyses wash off on the way to the completed slide. Enough remain to furnish cytologic details. In figure 1, F, 2 spermatia, *a* and *c*, have each secreted a small mass of dark-staining matter at the point of attachment to the paraphysis but have not started to enter. In figure 1, G and H, is an open passageway between spermatium and paraphysis. In G, the nucleus is moving in, and in H, *a, d,* transit is just completed. At *b* and *c* in figure 1, H, are what appear to be 2 other nuclei, and by focussing above the plane of the drawing, remnants of spermatia (not drawn) are found. In figure 1, I, is the remnant of a spermatium at *a;* at *b* is the introduced nucleus, now more expanded.

The irregular paraphyses formed under humid conditions also serve to receive spermatial nuclei (Fig. 1, C, *a;* D, *a*).

Under these excessively humid conditions and in the presence of mixed exudate, the spermatia on the leaf surface germinate in great numbers. Figure 1, J to N, shows details of germination of spermatia (×1400). The spermatium, at first elliptical, becomes cigar-shape (Fig. 1, J, K, L). On further growth, it may become more irregular (Fig. 1, M, N). The germ tube wall is very thin, and the contents stain lightly. So far as noted, it remains uninucleate and nonseptate. In figure 1, N, the germ tube started at *c*, grew to *a*, apparently met adverse conditions and the cytoplasm retreated and took a fresh start at *b*. Ordinarily, the germ tube remains unbranched.

Germinating spermatia are found also in mixed exudate at spermogonia. Their study has just been begun, but there are some indications that when paraphyses are young and vigorous, the spermatia fuse directly with them but when the paraphyses are old and decadent, the spermatia germinate and grow down through the ostiole into the spermogonium.

A more extended study of corn rust is in progress.—RUTH F. ALLEN, University of California, Berkeley, California.

BOOK REVIEW

Les Maladies à Ultravirus des Plantes. By Marie-Antoinette Beauverie. 175 pp., 8 pl. Reprinted from *Annales du Service Botanique et Agronomique de Tunisie.* Vol. 9, 1932. *Desvignes, Lyon.* 30 fr.

In summarizing the literature on the virus diseases of plants the author has faced a task of considerable magnitude and also of some difficulty, since the past ten years have witnessed an enormous increase both in the literature and in the complexity of the problem. Any attempt to condense this material into a paper of reasonable length is likely to result in occasional omissions and also in certain difficulties in the handling of conflicting evidence. The present work is not wholly free from such difficulties, but in view of the many discrepancies that appear in the literature itself, such omissions are not easily avoided. The very complexity of the problem, however, makes a work of this sort the more valuable, since it serves to bring the entire field of plant-virus research into at least an approximate focus. Mlle. Beauverie has accomplished this in very satisfactory fashion.

In taking up the subject the author has first defined the plant viruses on a basis of their characteristics, namely, filterability, ultramicroscopic nature of the causal agent, differentiation from filterable bacteria, biotropism, symptoms, and methods of transmission. Following this is a list of host plants based on the literature up to 1931 and a discussion of the wide-spread occurrence and the importance of control of these diseases. Chapter II deals with the technique of the study of the virus in extracted plant juices and furnishes a rather full discussion of methods of filtration and ultra-filtration as well as the effect of physical factors on the filtration process. It also covers the reactions of the viruses to heat, desiccation, dilution, adsorption, and other physical agencies, and the effect of chemicals and the possibilities of virus attenuation. The material is treated concisely and adequately and the discussion will prove of value to those who are interested in these phases of the problem. The third chapter presents the various theories regarding the nature of the virus and discusses the evidence on which these theories are based. Chapter IV gives a brief discussion of the distinction between infectious and noninfectious chloroses, and Chapter V deals rather fully with the pathological histology and cytology of plants affected by virus diseases. The material in this latter chapter is treated very satisfactorily, although it omits the work of Hoggan on the inclusion bodies associated with the different viruses affecting solanaceous plants and the value of such inclusions in the classification of certain of these viruses. Chapter VI takes up the transmission of plant viruses by insects and by artificial inoculation and the importance of soil, seed, and wild host plants

as sources of infection. This section might well have contained a more complete list of references on selective transmission by aphids in the case of certain combined virus infections, but it furnishes an excellent presentation of our knowledge of the transmission of these diseases. The same chapter deals with the variations in symptoms produced by a single virus on various hosts and the masking and alteration of symptoms that result from changes in environment. The methods by which the virus is thought to traverse the plant are also discussed.

Chapter VII describes the virus diseases affecting plants of economic importance, about one-third of the space being devoted to the virus diseases of the potato. This section has suffered somewhat from the condensed nature of the descriptions but it is generally adequate in its treatment. The discussion of tobacco mosaic, however, omits direct reference to the outstanding work of James Johnson on the virus diseases of his host and leaves the impression that the tobacco mosaics are due to a single virus. The statement that tobacco mosaic is transmitted by various aphids is also subject to qualification as a result of the work of Hoggan. Wheat mosaic and rosette are treated as distinct diseases, although the work of McKinney appears to have established the identity of these diseases. In making the above criticisms, however, it should be noted that each chapter is followed by a list of papers relating to the subject under discussion and that the omissions mentioned are generally included in these citations. Chapter VIII discusses the analysis of virus complexes by means of differential hosts, filtration, varying incubation periods, and selective transmission by aphids, while the concluding chapter outlines the analogies that exist between the plant and animal viruses. Following this is a bibliography of 762 titles, which is conveniently arranged and apparently very free from errors. Such a list of literature will prove of great value to all those interested in the subject, since there has been no previous compilation on such a comprehensive scale. The text also is admirably arranged for reference purposes, and any one who has reason to consult Mlle. Beauverie's work will agree that she has made a contribution of distinct value.—S. P. DOOLITTLE, Bureau of Plant Industry, Washington, D. C.

REPORT OF THE SEVENTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

OFFICERS

President ..... H. E. MORRIS, Montana State College, Bozeman, Montana.
Vice President ..... F. P. MCWHORTER, Oregon State College, Corvallis, Oregon.
Secretary-Treasurer ..... B. A. RUDOLPH, University of California Deciduous Fruit Station, San Jose, California.
Councilor ..... J. M. RAEDER, University of Idaho, Moscow, Idaho.

The annual meetings of the Pacific Division of The American Phytopathological Society were held jointly with those of the Pacific Division of the Botanical Society of America at the University of Utah, in Salt Lake City, June 14–15, 1933. The meetings were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies.

The effect of the world-wide depression was reflected in the poor attendance at the Salt Lake meetings, as a whole, but the meetings of the botanists and the phytopathologists were well attended, all things considered. Three half-day sessions were held, and as many as 50 members of the 2 societies were in attendance. The joint meetings in themselves proved an interesting variation from the usual routine, and the papers presented evoked considerable enthusiastic discussion. Abstracts of the papers concerned with plant pathology follow.

B. A. RUDOLPH, *Secretary-Treasurer*

ABSTRACTS

The dieback form of tomato streak.—M. SHAPOVALOV.

The term "streak" is a group name. Besides the combination streak, other forms have been reported. A very destructive form, locally known as "dieback," is prevalent on the Pacific Coast. The infection in some fields reaches 100 per cent. Affected plants, as a rule, become worthless. New shoots die back several inches and mosaic symptoms are characteristically absent, which is not the case with the combination streak. "Bronzing" is frequently seen.

Transmission of dieback by swabbing infective juices on healthy leaves is very difficult, whereas combination streak, under the same conditions, gave 100 per cent of infection. As revealed by biological differentiation tests, dieback virus does not contain either the green-tobacco or the latent potato virus. Of the 53 inoculated *Nicotiana tabacum* plants none developed symptoms, and retransfers to tomatoes gave negative results. Symptoms typical of the latent virus failed to develop on *N. glutinosa*. *Datura stramonium* occasionally developed mosaic without local lesions but with a tendency to leaf puckering; the inoculation of tomatoes with the juice from these Daturas failed to produce any disease.

Dieback symptomatologically is similar to "spotted wilt." The insect vector of dieback has not yet been discovered.

Some improvements in auto-irrigator apparatus.—L. A. RICHARDS and H. L. BLOOD.

To overcome the difficulty of preventing air leaks in prevailing types of two-piece double-wall irrigator pots a one-piece double-wall pot was constructed. Pots may be set

up for operation in groups by connecting them to a ¾-inch distributing pipe connected to an overhead reservoir that draws water from a constant-level supply reservoir through a connecting supply line. The air, which might leak into the system, passes to the overhead reservoir and may be removed with an aspirator pump. Single pots may be operated by equipping them with a supply tube leading into a supply reservoir and a modified thistle-tube reservoir on the respective arms of a ¾-inch "T" leading to the pot. The tension of the water in the pots is controlled by varying the water head or the amount of mercury inserted in the supply line. The capillary conductivity of the soil, the capillary conductivity of the porous-pot wall, the proportion of the soil to the water-supplying surface, and the rates of evaporation and of transpiration influence the precision of control obtainable with this system. The double-wall porous pot and modifications appear to have wide application in plant-disease investigations.

Some inoculations with Dothiorella ribis.—C. O. SMITH.

Inoculations through a wound on woody stems of plants with strains from the walnut, *Juglans regia,* and from the avocado, *Persea americana,* indicate that 25 species are susceptible and have lesions with a radius of 5 to 70 mm. The species successfully inoculated are as follows: *Citrus aurantium, C. grandis, C. limonia, C. sinensis* (most resistant); *Juglans regia, J. californica, J. Hindsii, J. sieboldiana; Carya pecan.* In the Rosaceae: *Cydonia oblonga; Eriobotrya japonica;* Rubus mammoth blackberry; Rosa Lady Hillingdon and Maman Cochet; *Prunus communis, P. domestica, P. avium; Pyracantha yunanensis, P. gibbsi; Annona cherimola; Carissia grandiflora; Ceratonia siliqua, Diospyros kaki, Eucalyptus* sp., *Feijoa sellowiana, Fraxinus ornus, Persea americana, Olea europaea, Psidium guajava, Salix* sp., *Ulmus campestris, Quercus lobata.*

Nine inoculations on *Juglans nigra,* six on Sapote, and four on *Pterocarya fraxinifolia* were negative.

Biochemical changes accompanying curly top of tomato.—F. B. WANN and H. L. BLOOD.

A comparative study of the biochemical characteristics of healthy and curly-top tomato varieties showed that the diseased plants were considerably higher than the healthy in dry matter and dry weight of pulp, sugars, and solids in the juice and in freezing-point depression of the juice. The pH of the juice was not changed appreciably by the disease, but the titratable acidity of the leaf juice decreased and of the stem juice increased. Catalase activity was lower in the diseased leaves. Total sugar increased and total nitrogen decreased in the diseased leaves, resulting in a higher C/N ratio. In the diseased stems the total sugars and starch increased, which the total nitrogen decreased in some varieties but increased in others.

A comparison of the biochemical characteristics of healthy varieties with the degree of resistance to curly top revealed that, in general, those varieties with higher values were less resistant than those exhibiting lower values for dry weight, solids, sugars, and freezing-point depression.

The effect of vitamins on the growth of fungi in pure culture.—W. G. SOLHEIM, S. S. SEARS, and R. C. ROLLINS.

Preliminary studies on the effect of vitamins B and G on the growth of *Helminthosporium sativum, Aspergillus niger, Penicillium* sp., and *Phyllosticta* sp. have shown that fructification is markedly increased when either vitamin is added to corn-meal agar. Similar effects were observed when potato-dextrose agar was used but they were not nearly so pronounced. *Helminthosporium sativum* grown on corn-meal agar, alone, produced a very loose growth, while with the addition of either vitamin a very dense growth

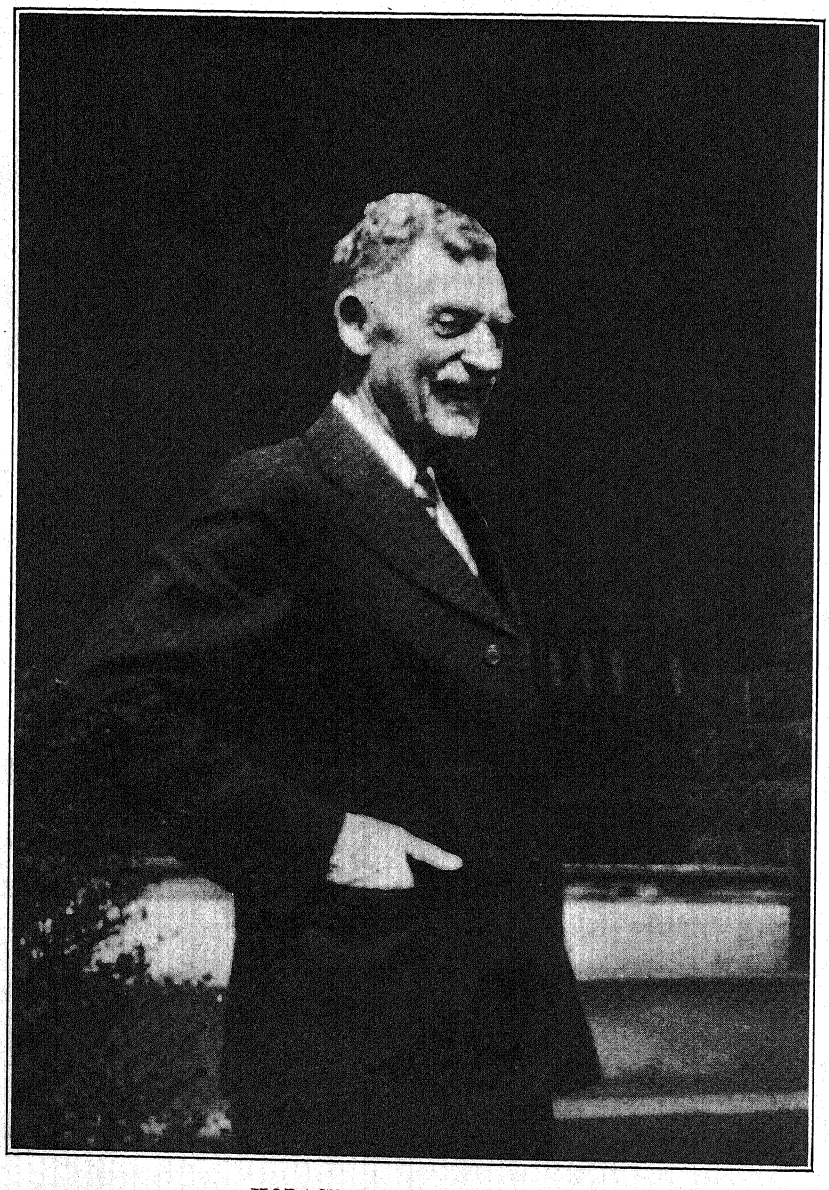
was produced. In the potato dextrose the same type of growth obtained throughout and was similar to that on the corn-meal agar plus vitamin. In the checks the conidia were borne in groups on a conidiophore, while they were borne singly on the agar + vitamin G. *Aspergillus niger* and *Penicillium* sp. produced a deep orange yellow pigmentation with vitamin. No pigmentation was observed in the checks.

Studies of psyllid yellows of tomato.—H. L. BLOOD, B. L. RICHARDS, and F. B. WANN.

Psyllid yellows of tomato is of immediate importance only in so far as it becomes a confusing element in curly-top investigations. The symptomatology of the two diseases is similar, thereby necessitating differentiation. While heavy losses from this disease sometimes occur in localized areas, rarely do such losses constitute a problem of major importance. The disease produces a puckering of the younger leaves followed by a dwarfing, a slight curling upward and purpling of the margins, a thickening and grayish discoloration of the laminae, and a purpling of the veins. Twisting of the petioles and rolling of the older leaves are sometimes pronounced. Curly top induces more intense yellowing and leaf curling and more rapid death than psyllid yellows. Inoculations with fewer than 30 insects have consistently failed to induce the disease. As all plants that have been fumigated for the removal of the insects following the development of the disease have recovered, it is apparent that continued insect feeding is essential to the continued expression of the disease. These results give additional support to the insect-toxin theory of the etiology of psyllid yellows.

New symptoms of psorosis, indicating a virus disease of citrus.—H. S. FAWCETT.

In May, 1933, on citrus trees affected with psorosis, a clearing of small areas in young leaves giving a mosaic-like appearance was noticed for the first time. These clear regions seem to disappear as soon as the leaf approaches maturity, but, in some cases, rounded, clear spots are formed on some of the older leaves. These spots on older leaves vary much in size from mere dots to areas 10 to 15 mm. in diameter and often are accompanied by a slightly raised brown surface, occasionally in the form of rings. The spots on older leaves have been observed by the author for a long time. Herbarium specimens dotted with small spots were collected November 6, 1923, from young orange trees 18 months after they had been budded from older trees showing psorosis. These same trees first showed the bark-scaling symptoms 8 years after budding. This condition is quite distinct from mottle leaf. The transmission of the disease with its previously known scaly symptoms on the bark and these new symptoms on the leaves has been demonstrated by budding and by rooted cuttings. It is now believed that the disease may occur either as a localized scaly-bark form on the bark, without any leaf symptom, or as a systemic form with both bark and leaf symptoms. It is also suggested that two other citrus diseases, leprosis and ring blotch, with symptoms on older leaves of the same general nature, should be investigated as to the possibility of their virus origin.



HORACE MANN WOOLMAN

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HORACE MANN WOOLMAN
1853–1932

HARRY B. HUMPHREY

Horace Mann Woolman was born in Damascus, Ohio, on December 23, 1853, and quietly passed from this life on December 23, 1932, in Orlando, Florida, at the age of 79 years. He had but a little while before gone to Orlando to escape the exactions of a northern Minnesota winter, and, as on similar occasions, was enjoying the kindly climate and peaceful atmosphere of that far-southern city, when, on the 16th of December, he was stricken down by paralysis from which he did not recover.

Mr. Woolman's forbears came to America with William Penn toward the close of the 17th century and settled in what was then known as Jersey. He was a direct descendant of a brother of John Woolman, the author of that early American philosophical classic, *The Journal of John Woolman,* and was, himself, a man endowed with the instincts of the student and scholar. True to the traditions of his Quaker lineage and background, Mr. Woolman was wholly and genuinely devoted to the philosophy of service, not for self but for the advancement and benefit of others.

Born at a time when, in the Middle West, facilities for acquiring a college education were extremely limited, it was not Mr. Woolman's privilege to enjoy such advantages until fairly late in life. Fired with an ambition to partake of the life intellectual, he availed himself of such educational facilities as there were in his boyhood environment and mingled with older men whose interests took them and their disciple afield into the realm of the "good life." Natural-born student that he was, he in time acquired a sufficient knowledge of civil engineering to qualify in that branch of applied science and he served in that capacity for several years. He it was who, while employed by the Chicago, Milwaukee, and St. Paul Railroad Company in the extension of its overland route through the mountains of Northern Idaho, surveyed one of the most difficult of the Coeur d'Alêne sections, and his recommendations were adopted. This was an accomplishment very much to his credit and one might, quite justifiably, have thought that, having gone thus far in his career in civil engineering, he would continue in its pursuit. But, even then, at the age of 56, the lamp of his unquenchable desire for the gifts of higher learning, still burning bright with enthusiasm and well-directed interest, we find Mr. Woolman registering at the State

College of Washington, where he pursued courses in botany, chemistry, bacteriology, and related subjects. The thing that through all his maturer years had been stuff for dreams and an insatiable longing was at last within his grasp, and he made the most of the opportunity. Incidentally, and with his characteristic, quiet enthusiasm he undertook also the study of the French and German languages. The upshot of this effort was that within a few years he had acquired a sufficient reading knowledge of both to enable him to abstract accurately such contributions to the literature on plant pathology as pertained to those research problems that were of peculiar interest to him.

Mr. Woolman, lacking a few credit hours of completing the requirements for college graduation, was appointed as field assistant in the Division of Cereal Crops and Diseases in 1917, with headquarters at Pullman, Washington. In that capacity he served ably until 1924, when, at the age of 70 years, he retired. So genuine was his interest in his chosen profession that for several summers after his retirement he kept himself informed and in touch with cereal-smut research, both here and abroad.

Toward the close of his career as a plant pathologist, at Pullman, and later at Corvallis and Moro, Oregon, Mr. Woolman acquired a working knowledge of the technique of plant breeding and applied himself effectively to the problem of the control of bunt through breeding bunt-resistant wheats. He produced a number of such varieties while in the service of the Department of Agriculture. In cooperation with Mr. David E. Stephens, he early investigated the susceptibility to *Tilletia tritici* of the 455 varieties included in the Department's wheat classification nursery at Moro, Oregon. Along with the varieties in this nursery, Mr. Woolman had under observation for resistance several selections from the Crimean group, a number of which were developed and have since shown marked resistance to many physiologic forms of *T. tritici*. The most outstanding of the sorts derived from Mr. Woolman's effort in this direction are the varieties Oro and Rio. The former is now being grown commercially in Southern Idaho. Segregating seed of hybrid strains from the cross Turkey × Florence were sent to Mr. Woolman by Dr. E. F. Gaines, of Washington State College. From this seed he developed a white-kernel variety, identical with the now well-known bunt-resistant Ridit, except for kernel color. This selection has since proved valuable as a parent in more recent crosses.

Later, Mr. Woolman made a number of wheat crosses between the bunt-resistant varieties Hussar and Banner Berkeley, Fürst Hatzfeld and Martin Amber, Hussar and Hohenheim 77, Ridit and Hussar, Martin Amber and White Odessa, Turkey and Florence, and a number of others. From these several crosses have come a few hybrid selections that have proved excep-

tionally resistant to bunt (*Tilletia tritici*), selections that may be especially valuable as parent material in subsequent breeding experiments.

It would be difficult to determine wherein Mr. Woolman made his most important contribution to our knowledge of bunt and its control. Suffice it to say that his excellent work speaks for itself; and those who were most intimately in contact with him, those who realized his devotion to research and caught his enthusiasm, were conscious of the value of his good work. The worth of the man is adequately expressed in these words, excerpted from a letter from Mr. David E. Stephens to the writer, written January 24, 1933: "I was distressed to learn of the passing of Mr. Woolman. I think few of us realized at the time he was carrying on his bunt investigations how much careful work he did and how much of his time and thought he devoted to his work. Mr. Woolman, I know, did his work thoroughly and carefully, devoting all his energy and capabilities to the problems he was investigating."

Those of us who knew Mr. Woolman best remember him as a man singularly free from prejudice, endowed with the graces of good-will, kindliness, and scrupulous consideration for the rights of others. Wholly free from any ostentation, a keen judge of human values, honest and honorable, he could not but endear himself to those whose good fortune it was to know him.

Mr. Woolman was, for several years, a member of The American Phytopathological Society and the American Association for the Advancement of Science. During the ten years of his research activity he contributed, in part or alone, the following papers:

Bunt or stinking smut of wheat. Wash. Agr. Expt. Sta. Bul. 126. 1915.

The prevention of wheat smut. Proc. Ann. Conv. Wash. State Grain Growers, Shippers and Millers Assoc. **10**: 45–49. 1916.

The wheat bunt problem in Oregon. Oregon Agr. Expt. Sta. Bul. 188. 1922. (With D. E. Stephens).

Cytological studies on the infection of wheat seedlings by *Tilletia tritici* (Bjerk.) Wint. (Abst.) Phytopath. **13**: 36–37. 1923.

Summary of literature on bunt, or stinking smut, of wheat. U. S. Dept. Agr. Dept. Bul. 1210. 1924. (With Harry B. Humphrey).

Studies in the physiology and control of bunt, or stinking smut, of wheat. U. S. Dept. Agr. Dept. Bul. 1239. 1924. (With Harry B. Humphrey).

Relative resistance of wheat to bunt in the Pacific Coast States. U. S. Dept. Agr. Dept. Bul. 1299. 1925. (With W. H. Tisdale *et al.*).

Infection phenomena and host reactions caused by *Tilletia tritici* in susceptible and non-susceptible varieties of wheat. Phytopath. **20**: 637–652. 1930.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

STOMATAL INFECTION WITH THE VIRUS OF TYPICAL TOBACCO MOSAIC

B. M. Duggar and Burt Johnson

(Accepted for publication March 10, 1933)

Incidental to extended studies of the physiological properties of the virus of typical tobacco mosaic, which are being carried on in this laboratory, it has seemed important to determine more precisely some of the possible avenues by which the virus may enter the plant when no wounds are apparent. Entrance of the virus through fresh wounds on leaves and stems has long been demonstrated and accepted. Whether these wounds are mechanical and artificial, such as mere abrasions, needle pricks, scratches, or cuts, or, as in the case of many other virus diseases, are the minute injuries made by certain sucking insects while feeding, there is "direct" penetration. Relatively few experiments seem to have been made with the idea of determining other possible means of entrance than through wounds. Allard (1) says, "Tests have also shown that simply pouring the sap of mosaic plants liberally upon the leaves of healthy plants is oftentimes sufficient to communicate the disease." Holmes (3, 4) has conducted extensive studies on types of injury on tobacco plants in relation to amounts of infection by the virus of tobacco mosaic.

In view of the great diversity of opinion regarding the nature of the virus agency, it is not indeed inconceivable that evidence for direct diffusion through uncutinized tissues might be sought by some investigators. Some work by one of us on the diffusion relations of this agency, to be published elsewhere, would seem to indicate that such efforts could yield only fruitless results. It may also be suggested at this time that the precipitation of the virus agent by fat solvents argues, on *a priori* grounds, against cuticular penetration, wholly aside from probable size considerations.

The possible entrance of the virus through stomata, however, has received far too little attention, and it is the purpose of this paper to report experiments bearing upon this means of entrance. It is highly important to determine to what extent stomatal infection may occur and whether or not a possible diurnal movement of the stomata of tobacco may have any influence on the entrance of the virus through the stomata, thus resulting in a greater percentage of infection in plants sprayed at one time of day as contrasted with plants sprayed at another time of day. If the stomata of tobacco exhibit marked diurnal movements, then differences in percentages of infection from hour to hour might be traced and correlated with differences in the stomatal apertures from hour to hour.

As a result of numerous studies on the part of many investigators, it is generally understood that stomatal movements of many plants are normally though perhaps indirectly controlled to a large extent by environmental conditions, particularly by light and humidity. The reasons for considering these effects "indirect" need not be developed in this paper. Temperature also is important, probably as much so because of its relation to light and humidity as to any more direct effect upon the plant. In general, there is abundant evidence to indicate that, while sensitive to light and other conditions, all the stomata of all species so far studied do not necessarily react exactly alike nor to the same degree to a given set of conditions.

Diurnal movements have, however, been largely correlated with light. Loftfield (6), working with a number of economic plants, found that "light induces the opening of stomata after daybreak," but that definite specific differences prevail. Humidity and temperature also were noted to have a marked effect on stomatal movements. On the other hand, there is evidence that the actual opening and closing of the stomata are in large part dependent on the species of plant studied. Loftfield noted such individual differences and Lloyd (5) previously furnished similar evidence on this point. Although Gray and Peirce (2) conducted their observations during daylight only, they found that the stomata of barley, wheat, oats, and rye are very sensitive to light, that the stomata are open widest when the light is most intense, and that they close when shaded, but there are some differences between species in this regard.

METHODS

The work here reported was done at different seasons. The experiments made during the winter were in a greenhouse, free from injurious insects, while during the summer an "insect-proof" tent out-of-doors furnished satisfactory semicontrolled conditions. Tobacco plants of Wisconsin Havana 142, a single selected strain of the Havana Seed type of tobacco, were used in all this work. The plants were carefully grown and at the time of inoculation (spraying) were in the 5-leaf or 6-leaf stage and were apparently free of wounds. A mulch of chopped cork was spread over the surface of the soil around the bases of the plants. The purpose of this $\frac{1}{4}$-inch layer of cork was to attempt to prevent infection through the roots by any of the virus suspension that might drain from the plants. However, very little of the sprayed material reached the soil, and if any should reach it and cause infections through the roots, such infections should be practically a constant factor. In one spraying experiment cork was not placed around the bases of the plants. The percentage of infection, however, was exactly the same as for those plants the roots of which were protected by

a layer of cork, but otherwise treated the same. All spraying experiments were so arranged that spraying of the various lots of a series was made each hour, or each 2 hours, or each 4 hours, according to the experiment, and extended through a continuous 24-hour period. Of course, in all operations great care was taken to see that the plants were under "normal" conditions and were not wounded or unduly disturbed at any time.

The suspensions of virus used for inoculations were prepared, unless otherwise noted, according to the standard methods employed in this laboratory.[1] Accordingly, the full strength, natural juice of diseased tobacco plants was diluted with distilled water to give 1:10 (1/10) concentration, and at this dilution the virus is freed of coarse impurities by diatomaceous earth (Celite). Controls were invariably employed to test the virulence of the 1/10, Celite-treated, infectious juice, which was used in spraying the plants. Control plants were inoculated by the needle-prick and scratch method. One drop of the infectious juice was placed in each of 3 locations on the plant; (1) in a leaf axil, (2) on the young stem, and (3) on a young leaf, each of these drops being immediately worked or pricked into the axil and scratched into stem and leaf blade with a sterile needle.

The spraying was carried out as follows: A De Vilbiss No. 15 atomizer was used to apply the virus to leaf surfaces in the form of a very fine spray. Both the upper and lower surfaces of the leaves were sprayed until the suspension began to form in drops on the leaves and run down the veins. The lower surfaces were sprayed by gently lifting the leaf with the nozzle of the atomizer and spraying while the leaf was so held. In this manner about 25 cc. of 1:10 Celite-treated, infectious juice were used on each set of 10 plants. A slight modification of this process for some of the experiments will be noted later. As soon as the spraying was completed, readings on temperature and humidity were taken, using for the latter a sling psychrometer. Also, at the same time, a strip of the upper epidermis from a single leaf, one from each of 2 plants (not used for inoculations), was removed and both strips were immediately plunged into a vial of absolute alcohol, these samples being taken for the purpose of determining the condition of the stomata in respect to size of stomatal opening. Using the same two plants, but different leaves, strips of the lower epidermis were removed and placed in a separate vial of absolute alcohol. This whole process (spraying, taking data on temperature and humidity, and removing strips of epidermis) on the average required 3 to 6 minutes, mostly about 5 minutes.

In order to carry out the above-mentioned procedures at night and still use as little light as possible, the following methods were used. The psy-

[1] Duggar, B. M. Proc. Soc. Exp. Biol. Med. 30: 1104–1109. 1933.

chrometer readings were started while the lights were still out. Then a small frosted bulb at one side of the greenhouse was turned on, and the readings on temperature and humidity were taken, and also the spraying of the 10 plants for that hour was done by this weak light. The small light was then turned out. The samples of epidermis were taken from plants distant from the weak source of light, so that even this brief illumination would have little effect on the plants from which the epidermis was taken. Over these plants was placed a large frosted bulb and the light from this bulb was used only for 30 to 45 seconds while securing the specimens of epidermis. For night work in the insect-proof tent, an ordinary oil lantern furnished light for all operations.

The samples of epidermis were stored in the alcohol until examined, when a saturated solution of Congo Red in absolute alcohol was substituted for the absolute alcohol used as the killing agent. The stained strips of epidermis were, when prepared for final study, mounted in absolute alcohol and examined under a microscope provided with an eyepiece micrometer, so that measurements of pore widths might be made. Since the idea was to get a general picture of the condition of the stomata of the tobacco leaf under the conditions of these experiments, each sample was studied carefully under high-dry magnification, observing a large but not fixed number of stomata—often as many as several hundred, varying with the size of the sample. By careful estimation and by checking measurements from time to time, the stomata were divided into 5 groups as follows: (1) pores entirely closed, (2) pores open to 2.5 μ across, (3) pores 2.5–5 μ across, (4) pores 5–7.5 μ across, and (5) pores more than 7.5 μ across.

The procedure of stomatal study used in these experiments follows quite closely that of Lloyd (5) and of Loftfield (6) and has been discussed in full by them. The method for the determination of stomatal size is open to criticism on a strictly quantitative basis. However, the purpose of this work was not to get absolutely accurate information about the stomata as such, but rather to secure information that might disclose any possible correlation between (a) size and movements of stomatal pores and (b) incidence of infection resulting from the spraying. For such a determination, all that is necessary is to secure a relative, quantitative expression of the characteristics of the stomata. By no other method, perhaps, could such a large mass of material be examined and such a general quantitative idea of the tobacco stomata be obtained. Besides, all conditions of the cultures, including environment and variety of host selected, are never exactly reproducible, in the hands of different investigators, so that it would be rather futile to set down absolute measurements.

In reference to the spraying technique, it could be objected that the mere impact of the spray particles might break the epidermal hairs, per-

mitting the entrance of the virus through wounds. However, even a casual examination of the resistance of these hairs would be against this interpretation; furthermore, we have been unable to find any evidence of injury from the use of a De Vilbiss atomizer. It should be noted, too, that no infestation of insects nor occurrence of wounds made by insects was observable on the experimental plants. That some slight wounds may occur on plants grown with the greatest care is granted, but the rôle of such wounds in infection requires demonstration, and experiments will be described later in this paper bearing upon this point. The possibility of root infection is remote and will also be considered in a later paper.

EXPERIMENTAL WORK

Preliminary work had indicated that under the conditions prevailing in the greenhouse used, infections could be obtained to the extent of 80 per cent of the plants sprayed, using the typical tobacco mosaic virus. Duggar (unpublished data), while working at the Missouri Botanical Garden, had secured a considerably lower percentage of infection by spraying full-strength, infectious juice (previously filtered through a porous porcelain filter) on healthy plants. He obtained under the conditions of those experiments 30 to 50 per cent of infections but was not satisfied with the technique employed nor with the control of the experiments.

Experiment I was a series of samplings of epidermis and of inoculations by spraying under reasonably controlled and recorded greenhouse conditions. The technique was essentially as outlined and discussed above. The experiment was started on April 2 at 4 p. m. and closed at 4 p. m. on April 3. Both days were bright and clear. Ten new plants were sprayed each hour throughout the 24-hour period. The control inoculations were made as explained above. Control *X* was inoculated at the beginning of the experiment—4 p. m., April 2. Control *Y* was made in exactly the same way and from the same lot of material, except that the plants were inoculated at the close of the experiment—4 p. m., April 3. The controls here were intended to test the virulence of the suspension of virus used in the whole series. In both cases the incidence of disease in the controls was 100 per cent.

Considering for the moment only the disease frequencies resulting from the 24 lots of sprayings, it will be seen (fig. 1, A) that the infections from this method of applying the inoculum are relatively high, averaging approximately 70 per cent; the range is 30 to 90 per cent, and in only 2 periods is it less than 50 per cent. It is equally important to note that there seems to be no discernible correlation between time of day or environmental conditions and the percentage of disease. The 2 low points in the infection curve, which appear in the daylight intervals, may be regarded for the

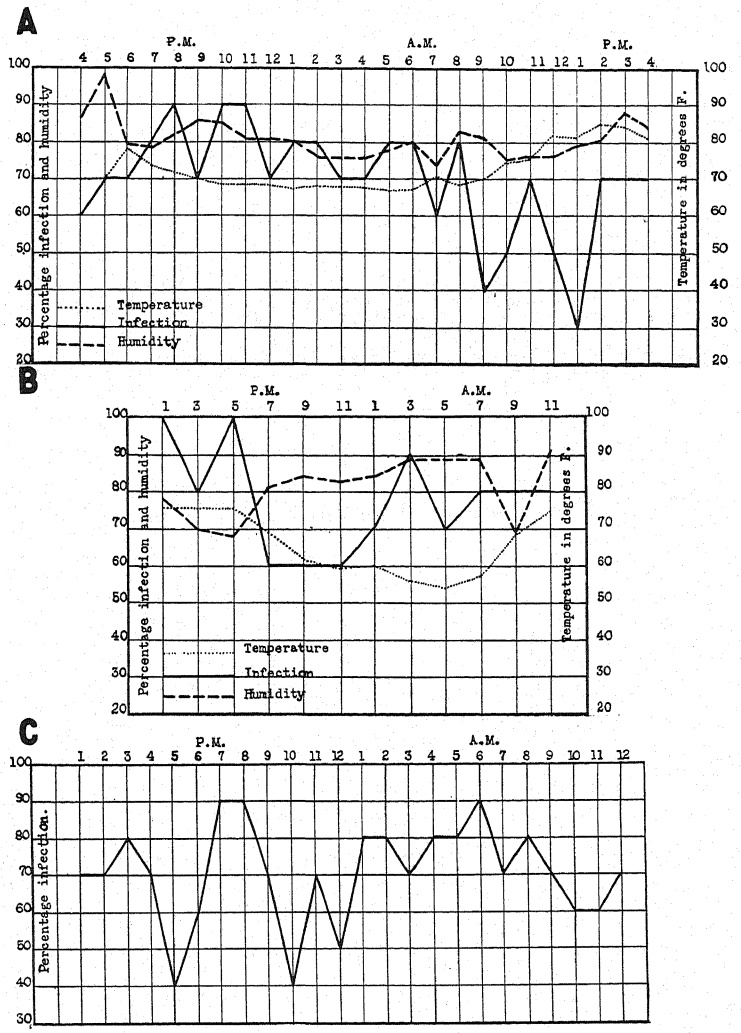


FIG. 1. Physical conditions at the time of and results of spraying tobacco plants with the virus of typical tobacco mosaic at intervals during 24-hour periods on different dates. A. Hourly in a greenhouse on April 2 and 3. B. At each 2-hour interval in an "insect-proof" tent on August 3 and 4. C. Hourly in a greenhouse on October 4 and 5. Apparently plants were not mechanically injured during the spraying.

moment as accidental. The relation of the various intervals to stomatal opening will be considered later.

Experiment II was carried out in the shade tent[2] on August 3 and 4.

[2] The plants were potted in the ordinary manner in the greenhouse and were allowed to remain there until well established, then they were transferred to an insect-proof tent and were allowed to grow there until they had reached the size generally used in this laboratory for inoculation, namely, the 5-leaf and 6-leaf stages.

This experiment was conducted exactly as was the one in the greenhouse, with these 2 exceptions: (1) Inoculations were made every 2 hours during a 24-hour period instead of every hour. (2) Night work was done by lantern instead of by electric light. The weather was partly cloudy. Although growing conditions in general differed considerably from those in the greenhouse, the temperature in the tent at the time of these sprayings was much the same as for the previous experiment, and the humidity in the tent was lower during about half the time. (Fig. 1, B.) Nevertheless, the curve of infection percentage is apparently as unrelated to environmental conditions as in the preceding experiment. Moreover, in the present experiment the 3 low points in the curve fall in the night interval. The controls again exhibit an incidence of 100 per cent diseased.

The data on the sizes of stomatal apertures in the 2 experiments are extensive. Since these data are somewhat simplified by the 2-hour intervals of experiment II, they will be given in detail for this series (Table 1). It will be noticed that there is a surprising variability in the extent of stomatal openings, and at no period of either day or night is there complete opening or complete closure of the stomata. Certainly, one would be disposed to draw the general conclusion that there are no striking correlations between width of stomatal openings of the tobacco thus grown and such environmental factors as temperature, humidity, and light, nor between width of stomatal openings and percentage of infection. However, a closer analysis of this last relation will be made, although it is fully realized that many other factors may affect the percentage of infection.

Assuming that infection may be related to extent of stomatal openings, the only possible method of analysis would seem to be that of summing the percentages of stomatal openings at any one time-period in each of the 4 size-classes separately (omitting, of course, the "closed" size-class), and then weighting these classes by multiplying by the proper factor, according to the areas involved in each class. Since the figures given in each line of the table represent 4 samples, 2 of the upper surface, and 2 of the lower surface, an average of each class may be taken, being less cumbersome. Then, a summation of the results thus weighted will give a figure that may be called the "infection probability coefficient."

The 4 classes referred to are (a) up to 2.5 μ, (b) 2.5–5.0 μ, (c) 5.0–7.5 μ, and (d) above 7.5 μ, in width, and for these classes it has been found that for all practical purposes it is satisfactory to weight these, respectively, as 1, 2, 3, and 4. This is based on the consideration that the stomatal pores are ellipses, and the area of an ellipse = πab, where $a = \frac{1}{2}$ the short diameter and $b = \frac{1}{2}$ the long diameter.

On this basis the coefficients from table 1 are found to range from 271 to 77.5, the averages being as follows: 100 per cent infection = 258; 90–70

TABLE 1.—*Data showing the percentages of stomatal pores falling into different-size groups, the temperature in degrees F., the relative humidity, and the percentages of infection due to spraying with the virus of typical tobacco mosaic at each two-hour period during a day. (Ten plants sprayed each period with 1:10 Celite-treated infectious juice.) Experiment arranged in an "insect-proof" tent, sprayings on August 3 and 4*

Time when stomata were open	Percentage of stomatal pores falling in the different-size groups																Temp. °F.	Humidity %	Infection %
	Upper surfaces of leaves										Lower surfaces of leaves								
	Leaf No. I					Leaf No. II					Leaf No. I					Leaf No. II				
	Figures in microns					Figures in microns					Figures in microns					Figures in microns				
	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5			
1 p. m.	0	0	20	80	0	...	...	...	...	...[a]	0	0	0	20	80	0	50	50	0	0	76	78	100
3 p. m.	0	40	30	30	0	5	35	50	10	0	0	0	25	60	15	0	0	15	60	25	76	70	80
5 p. m.	...	...	...	...	...	...	...	...	...	...[a]	0	10	10	30	50	0	45	40	15	0	76	68	100
7 p. m.	10	50	40	0	0	30	70	0	0	0	40	60	0	0	0	50	50	0	0	0	69	81	60
9 p. m.	10	70	20	0	0	80	20	0	0	0	0	15	25	40	20	60	30	10	0	0	62	84	60
11 p. m.	20	80	0	0	0	20	60	15	5	0	0	50	50	0	0	0	40	60	0	0	59	83	60
1 a. m.	50	25	25	0	0	30	50	20	0	0	0	5	10	35	50	5	90	5	0	0	60	84	70
3 a. m.	0	50	35	15	0	20	80	0	0	0	0	50	30	20	0	0	45	50	5	0	56	88	90
5 a. m.	40	40	20	0	0	35	60	5	0	0	40	55	5	0	0	40	55	5	0	0	54	88	70
7 a. m.	0	50	50	0	0	0	30	40	30	0	0	15	25	50	10	5	15	50	15	15	57	88	80
9 a. m.	10	40	50	0	0	0	50	50	0	0	0	10	50	40	0	15	20	55	10	0	69	68	80
11 a. m.	5	90	5	0	0	10	90	0	0	0	0	20	80	0	0	0	50	50	0	0	75	91	80
Average incidence of disease in per cent																							77.5
Control *X* (1 p. m.)																							100
Control *Y* (11 a. m.)																							100

[a] No readings made.

per cent, 155.9; 70–60 per cent, 115.3; 60 per cent, 104.2. These figures are at least suggestive of a relation between stomatal openings and percentage infection. Unfortunately, the greenhouse series is not so consistent. Nevertheless, even in that series, it is true that the higher percentages of infection have high coefficients, but not all of the lower percentages of infection exhibit correspondingly low coefficients.

In order to determine the influence of a more prolonged period of darkness on stomatal behavior in tobacco and on incidence of infection, 10 plants were placed in a dark room for 48 hours and then sprayed with 1/10, Celite-treated, infectious juice, and, after spraying, were allowed to remain an additional 12 hours in the dark room. While the plants were in the dark room they were well watered. Of these plants 60 per cent became diseased. At the end of the 48-hour period samples of epidermis were taken and later examined as previously described. Examination of the stomata showed percentages falling in the several size groups, as exhibited in table 2. Thus, it

TABLE 2.—*Percentage of stomatal pores falling into different size classes, in tobacco, after the plants were held for 48 hours in a dark room*

Leaf surface	Leaf No. I			Leaf No. II		
	Open to 2.5 μ	2.5 μ to 5 μ	5 μ to 7.5 μ	Open to 2.5 μ	2.5 μ to 5 μ	5 μ to 7.5 μ
Upper	50	50	0	50	50	0
Lower	40	40	20	10	40	50

is seen that in even prolonged absence from light, the stomata are at least partially open.

It should be noted that final observations to determine the amount of infection were made in all cases 3 weeks after spraying, since, in our experience, tobacco plants grown under ordinary conditions will express symptoms of mosaic, if present, well within this period in the usual infection experiments.

Owing to the possibility that the method of inoculation had resulted in wounding the plants and that the infections had occurred through such possible wounds, other spraying experiments were arranged in the hope of evaluating this possibility. It might conceivably be possible that lifting the leaves of the plants with the nozzle of the atomizer, in order to spray the under surfaces of the leaves, might cause injuries, and, if so, a mechanical wound inoculation of the plant might be made instead of an intended spray inoculation.

Experiment III, therefore, deviated from the practice for spraying as outlined under the heading "Methods," in that only the tops of the leaves

were sprayed. At no time were the leaves touched by the operator or by the atomizer. No cork was placed around the bases of the plants in this experiment. Nevertheless, there was no increase in the percentage of infection. This fact rather definitely indicates that the infections observed in these spraying experiments were not the result of virus penetration from the soil by means of the roots. Otherwise, essentially the same conditions prevailed in this experiment as in experiment I. Figure 1, C, graphically indicates that the experiments are definitely of the order of constancy observed in those already described, although in this latter work even stricter precautions were observed in the prevention of injury.

In still another experiment, using approximately 100 plants (but younger) in each series, and spraying each 4 hours during a 24-hour period, results comparable to those seen in table 2 were obtained. In this experiment also, the plants were not touched by the operator nor by his instruments. It would seem then that when this virus is sprayed on plants, entrance through stomata must occur, since there has been a strict control of the injury possibility. Nevertheless, it is essential to determine the effect of injured epidermal structures on the incidence of infection.

In order to test the relation of injury and noninjury of the epidermal hairs to incidence of infection, experiments were performed under 5 different environmental conditions. These experiments and the results obtained are briefly presented in table 3. Further explanations of (a) the

TABLE 3.—*Results showing the percentage of infection caused by dropping the virus of typical tobacco mosaic on injured and uninjured leaf surfaces, using plants somewhat differently treated. Numbers are the percentages of infections based on the use of 10 plants in each test. "C.T.I.J." signifies "1: 10 Celite-treated infectious juice"*

State of the leaves inoculated	Treatment of experimental plants				
		In insect-proof tent cage			
	A. Potted plants + C.T.I.J.	B. Potted plants + C.T.I.J.	C. Wilted potted plants + C.T.I.J.	D. Potted plants + natural infectious juice	E. Older plants in natural soil + C.T.I.J.
(1) Upper surfaces intact	30	20	10	40	10
(2) Lower surfaces intact	10	0	0	50	0
(3) Upper surfaces injured	20	100	70	100	40
(4) Lower surfaces injured	60	70	60	90	40
(5) Control–needle-pricks	70	100	100	100	100

state of the leaves inoculated (designated by the numerals 1–5 in table 3) and of (b) the general environmental conditions, together with sources of inocula (designated by the letters A–E in table 3), are necessary and are given in the correspondingly numbered and lettered paragraphs below.

(1) Each plant inoculated received 3–5 drops of the inoculum dropped upon the upper surfaces of each of 2 leaves. The part of the leaf supporting the virus suspension was apparently intact and uninjured.

(2) Precisely the same as in (1), except that the lower surfaces of the leaves received the drops of inoculum.

(3) Precisely as in (1), except that before dropping the suspension of virus on the leaves the upper surface of each leaf "inoculated" was vigorously and thoroughly brushed over with a camel's-hair brush, this treatment resulting in apparent injury of epidermal hairs.

(4) The lower surface of each of 2 leaves of each plant in the test treated exactly as described in (3).

(5) The control plants (10 in number, as in each block above) were inoculated by our standard needle-prick method, that is, pricking or scratching into the tissues a few (3) drops of the virus suspension indicated.

The further notes on environmental conditions, not adequately described in table 3, are as follows:

A, greenhouse-grown, early spring. B, tent-grown, midsummer. C, tent-grown, midsummer; the plants were deprived of water for 42 hours prior to inoculation. After the suspension of virus had dried on the leaves, the plants were watered and soon appeared normally turgid. D, tent-grown, midsummer. The suspension of virus (1:10 natural infectious juice) was not treated with Celite in this experiment. E, tent-grown, midsummer. The plants were growing in the field soil over which the tent was built. As these plants (Group D) were quite old, they were cut back to yield new shoots, and they were inoculated one week after topping.

From the results in table 3, lines (1) and (2), it will be seen that some infections may result from dropping the infectious juice on the apparently uninjured surfaces of leaves. This method is, however, rather erratic and is doubtless dependent to some degree, possibly to a considerable degree, upon the "spread" of the drop on the leaf.[3] The amount of leaf covered is less, of course, than when a spray is employed, and the percentage of infection averages considerably less. As would be expected, the freshly injured leaf surfaces, table 3, lines (3) and (4), afford a high percentage

[3] In this connection it should be noted that in some earlier experiments in which drops of diluted infectious juice were placed on tobacco leaves the incidence of infection amounted only to an occasional diseased plant. One of these experiments was made in cloudy weather, the plants were exceptionally turgid, and the immediate spreading of the drops was insignificant.

of infections, the older plants exhibiting, in general, the lowest average. Another factor requiring consideration is that of the "age" of the wound. Our experiments confirm, in general, the findings of Holmes to the effect that the higher incidences of disease are obtained when the inoculating needle carries the virus with it, or when inoculation consists in pricking through a drop of the virus suspension. Commonly, placing drops of the virus on old wounds is far less effective than a similar treatment of fresh wounds. Returning then to a consideration of our sprayed plants, it may be deduced that with the precautions taken to avoid fresh wounds, and, wholly aside from the duplication of controlled features already referred to, the existence of old wounds would not seem to constitute an important factor in disease production.

Since it is clear that a high incidence of disease results from spraying healthy, young tobacco plants with infectious juice and since the probability seems to be established that stomatal penetration of the virus can well occur, some explanation of the whole phenomenon of infection should be available. There are then 2 aspects of this problem, (1) the entrance of the suspension into the substomatal cavities and (2) the mechanism of penetration into the cells. The first-mentioned is amenable to experimental study and will be considered first, although at this time our observations are merely preliminary. If a glass slide or Cellophane sheet is swung rapidly through a spray of greatly diluted India ink the spray droplets on hitting the target will be found to have taken the form of ellipsoidal films. We have measured these films but have not measured the droplets. We have estimated these droplets, on the basis of the character and small diameter of the films, to be approximately one-third the diameter of the latter, the smaller droplets probably 10 μ in diameter. It is possible that such droplets might be shot through the fully open stomata; but there seems to be a far greater likelihood that films formed as the smallest droplets strike the inner edges of the guard cells would be forced through the opening, just as the long-ellipsoidal films are produced on the moving glass or Cellophane.

In order to make an approximate test of the entrance of suspension droplets through the stomata, an alcoholic solution of nigrosin and an aqueous suspension of India ink were prepared and each was sprayed on a separate series of 10 plants each. After evaporation of the liquids, samples of the epidermis were secured and fixed in absolute alcohol. Microscopic examination of these samples showed, in the case of the India ink, that (a) large blotches of the ink adhered to the surfaces of the leaves and that (b) from 3 to 5 per cent of the stomata not covered by the blotches were wholly or partially filled by the ink. This observation was confirmed by a later test in which the presence of the India ink in some of the sub-

stomatal cavities was readily identified but no quantitative relations established. The nigrosin, of course, was dissolved by the fixing alcohol but, nevertheless, enough of the particles remained in a few of the stomata to suggest at least a considerable penetration of this material, as well.

In reference to the mechanism of penetration into the living cell, this problem is wholly hypothetical and will be considered more at length in a later paper. It may be said, however, that the senior writer has long assumed a dominant rôle of protoplasmic bridges, or plasmodesma, in the transfer of the virus from cell to cell. For the moment, then, the question may merely be raised as to whether or not interfacial tension may be adequate to insure the "creeping" of the virus particles between the walls of cells of the mesophyll to points where adsorption or picking up of the particles by the protoplasmic fibrils might take place.

It seems rather remarkable that up to the present we have found the incidence of infection very low when leaves are partially immersed in a suspension of virus. In one of the early experiments in which this was true the suspension was confined by a ring of white vaseline, the leaves treated being supported horizontally. It was determined to elaborate somewhat this type of experiment, and the series outlined below was arranged, yielding infection results as indicated in each case. Ten plants were used in each of the 7 experiments referred to, a vigorous, attached leaf in each case (except in the control) being immersed, by tilting, in the suspension described. (1) One leaf of each plant was largely immersed in a vessel of 1/100 C.T., infectious juice, remaining 3 weeks. Result: 1 plant diseased. (2) Same as in (1), except the tip of the leaf was excised with sterile scissors before immersion. Result: 1 plant diseased. (3) A healthy leaf of each plant was immersed, as in the preceding, and then the entire set-up placed in a partial vacuum, amounting to 11 cm. of mercury, for 10 minutes; then, after removal from the partial vacuum, the plant was returned to growing conditions, the leaf remaining partially immersed for 3 weeks. No water-soaked areas developed. Result: 1 plant diseased. (4) Same treatment as in (3), except that the leaf was removed from the virus suspension after the partial-vacuum treatment. Result: 2 plants diseased. (5) Same treatment as (3), except that prior to treatment in the partial vacuum the leaf tip was excised with sterile scissors. Result: 1 plant disease. (6) Treated as in (4), except that the tip of the leaf was excised before the partial-vacuum treatment. Result: 3 plants diseased. (7) Control, needle-prick inoculation, with the same 1/100 C.T.I.J. Result: 8 plants diseased.

From these results two suggestions may be made: First, dipping a leaf into a requisite volume of water is not comparable with spraying or other

similar procedure, and it seems necessary to infer that surface-tension relations are not the same.

On the other hand, the presence of any important "natural" wounds or of those induced in the plants of experiments (2), (5), and (6), described above, would seem to offer avenues of entrance, if such wounds were highly effective for virus penetration. The second suggestion is that the plants of these experiments required more "manipulation" than those sprayed with the virus suspension, and, since no significant percentage of disease results in these immersed plants, we may feel relatively certain that manipulation and corresponding injury did not constitute a significant factor in the incidence of disease obtained by spraying.

It should be pointed out that as a result of spraying the suspension on leaf surfaces there are formed relatively vast spreading films, as well as droplets. We do not yet know what the condition of the surface is when immersion occurs; possibly the stomata are blocked by air bubbles.

The various experiments and observations reported in this paper seem to establish with reasonable probability the view that stomatal infection may occur when a suspension of the virus of typical tobacco mosaic is atomized or sprayed upon the young leaves and shoots of the tobacco plant.

SUMMARY

In this paper there is presented a discussion of the technique of spraying tobacco leaves with a virus suspension in the effort to determine whether or not stomatal infection may occur. In respect to this infection problem a study has been made of stomatal behavior in the tobacco plant during 24-hour periods in 1-hour and 2-hour intervals in relation to such factors as light, temperature, and humidity. No significant correlation has been detected between the number of stomata open nor the extent of opening and these environmental factors. At no observation-time were all the stomata on a leaf closed.

The average incidence of infection from spraying is significantly higher than previous experiments had indicated. Cognizance has been taken of the possibilities of entrance of the virus by means other than through the stomata, but the evidence deduced is opposed to such possibilities. Some degree of correlation may be found between percentage of infection and the extent of stomatal opening, but no correlation is found between percentage infection and environmental factors within the range of the experimental conditions.

Dropping a virus suspension on regions of the leaf where epidermal hairs had been extensively injured by rubbing did not increase the amount of infection over that induced by spraying with the suspension. On the

other hand, placing large drops of virus suspension on uninjured leaves induced a lower percentage of infection.

A very low incidence of infection occurred when individual, vigorous, leaves attached to the plants were submerged for varying lengths of time in suspensions of the virus, and attention is directed to surface-tension effects.

It is suggested that the atomized particles of virus suspension may be directly "shot" into fully open stomata, but the probability is greater that the spreading of those films formed as the spray particles impinge on the walls of the open stomata is a chief means of entrance. A problem of much significance is the mechanism of penetration of the virus particle into the living cell from the substomatal cavity, since this may be related to the problem of virus movement from cell to cell.

UNIVERSITY OF WISCONSIN,
MADISON, WIS.

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SEEDLING STEM BLIGHT OF FIELD BEANS CAUSED BY RHIZOCTONIA BATATICOLA AT HIGH TEMPERATURES[1]

JAMES B. KENDRICK[2]

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Following periods of unusually high temperatures, a destructive seedling disease of Red Mexican, Red Kidney, and Pink beans, *Phaseolus vulgaris* L., caused by *Rhizoctonia bataticola* (Taub.) Butler, was observed in 1929, 1931, and 1932 in the Sacramento and San Joaquin valleys of California, where beans for dry-bean production are grown during the dry hot summer months. Infection occurs near the cotyledonary node and usually kills the seedlings. The fungus was consistently isolated from the lesions and its pathogenicity to seedlings at high temperatures proved in inoculation tests.

The rather wide-spread occurrence of this fungus in California on maize, beans, and cowpeas in the mature stages of these crops has recently been reported by Mackie (5). The disease of beans caused by this fungus, or closely related forms, has been found in many parts of the world, particularly in tropical regions. Stem infection of jute and cotton seedlings by this fungus, then designated as *Rhizoctonia solani,* was found by Shaw (7) in India and in inoculation tests he obtained infection of jute, cotton, groundnut, and cowpea seedlings. He later (9) reported the fungus on *Phaseolus lunatus.* Briton-Jones (2) found the fungus causing a root rot of beans and cowpeas in Egypt and obtained infection of seedlings of both host species. Small (10) found the fungus on beans in Uganda, obtained infection of bean seedlings, and later (12) observed the organism on beans in Ceylon.

In the United States, the ashy stem blight of beans reported from South Carolina by Ludwig in 1923 and 1925,[3] from Georgia by Boyd[4] in 1927, and from Mississippi in 1926 by Wedgworth (17) is caused by *Macrophomina phaseoli* (Maubl.) Ashby, which the work of Shaw (8), Ashby (1), and Haigh (4) has shown to be a pycnidium-producing strain of *Rhizoctonia bataticola.* J. T. Barrett observed this disease of beans at Riverside, California, in 1919. This fungus has also been found on beans in Palestine by

[1] Contribution from the Division of Plant Pathology, Branch of the College of Agriculture, University of California, Davis, California.

[2] The writer wishes to acknowledge the helpful advice of Professor Ralph E. Smith and the assistance of Dr. Max W. Gardner in the preparation of the manuscript.

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[4] U. S. Dept. Agr. Bur. Pl. Industry Pl. Dis. Rptr. 10: 18. 1926. (Mimeographed.)

Reichert and Hellinger (6), who obtained infection of bean seedlings in inoculation tests.

SEEDLINGS KILLED AND STAND REDUCED

The damage caused by the disease is primarily due to irregularity of stand and subsequent reduction in yield. If environmental conditions favor its development, many young plants are killed as they emerge, or shortly after they emerge, from the soil. Diseased plants quickly dry up and are hidden by adjacent healthy plants or are covered with soil during cultivation. The grower in many cases does not recognize the trouble as a disease and attributes the poor stand to poor germination or to hot-weather injury. It is only in extreme cases, *i.e.*, where a high percentage of the plants are killed, that alarm is caused. There is sufficient evidence to indicate that in certain seasons, notably those in which high temperature prevails when the plants are in the seedling stage, serious damage to the bean crop in interior California has been caused by the disease.

Seedling stem blight was first observed by the writer on July 25, 1929, in a 40-acre field of young Red Mexican beans near Lodi, in the San Joaquin valley. The many irregularities in stand and presence of dead plants indicated that the disease had been present in the field for at least 2 weeks. Approximately 50 per cent of the plants were dead or dying and diseased plants occurred more or less uniformly throughout the field. An intensive survey of the bean plantings in the surrounding area revealed the presence of a similar disease in many fields where the plants were approximately of the same age as those in the field noted above. The severity of the disease varied from a trace to more than 10 per cent. Scattering cases of the blight were observed and many short skips or irregularities in stand indicated that the disease had been more severe earlier in the season. One could find the small, dried-up, diseased plants when the soil that had been piled up around the plants by the cultivator was removed. Careful inspections of the fields in which the blight occurred in July were made during the remainder of the season and the disease was not observed on plants that had escaped infection in the seedling stage. A survey of the large bean-producing area in the upper delta region immediately west of the Sacramento River failed to show the presence of the blight. No cases of the disease were found in the extensive Blackeye cowpea plantings of the San Joaquin Valley.

The blight appeared again in 1931 on seedling beans throughout the Sacramento Valley. In some fields, the stand was reduced from 10 to 20 per cent and certain areas in these fields showed a 50 per cent mortality of seedlings. In some plantings many of the young seedlings were killed before reaching the surface of the soil. It was again observed that the plants that had escaped the disease in the seedling stage did not become infected. It was not found in plantings of Henderson's Bush Lima beans.

On July 13, 1932, an examination of 600 acres of Red Kidney beans was made near Orland, in the upper Sacramento Valley, where blight was found to be severe over the entire planting; approximately 60 per cent of the young plants were dead or dying. The following day the disease was found to have reduced the stand from 30 to 40 per cent in a field of young beans near Lodi, in the upper San Joaquin Valley.

FIELD OUTBREAKS FOLLOW PERIODS OF HOT WEATHER

The sudden appearance of seedling bean blight in 1929 followed an extended period of high temperature. Practically every field where the disease occurred had been planted immediately before or during a period of high temperature extending from June 21 to July 5. The daily mean temperature recorded by the U. S. Weather Bureau at Stockton, not far from Lodi, was near or above 80° F. during the above named period (Fig. 1).

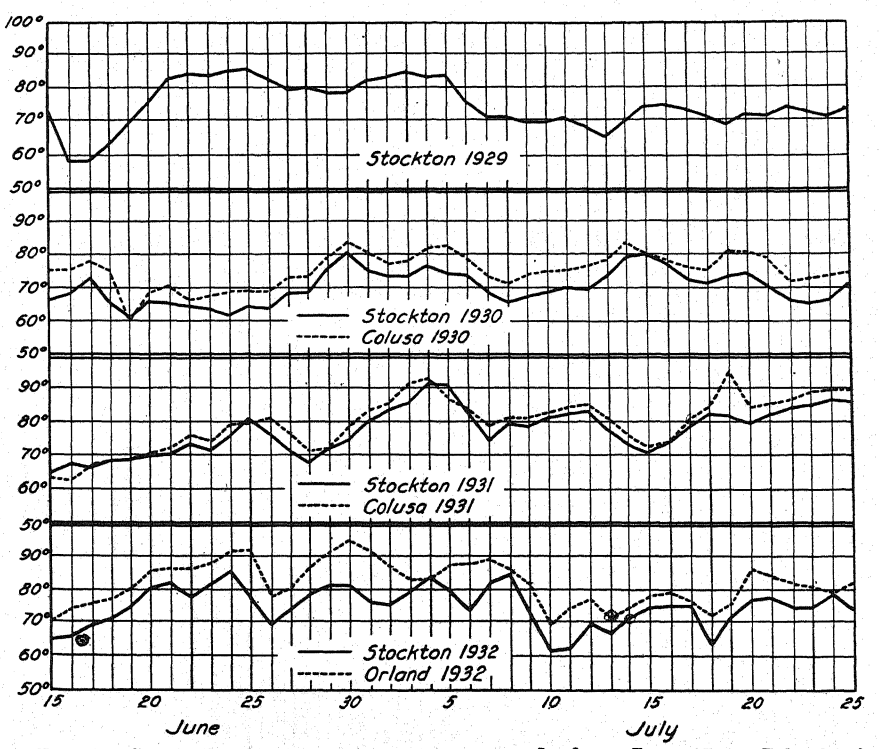


FIG. 1. Graph showing mean temperature records from June 15 to July 25 for Stockton, 1929 to 1932; Colusa, 1930 and 1931; and Orland, 1932. During this period most of the beans are planted in the valleys of central California where epiphytotics of seedling stem blight have been observed following prolonged periods of a mean temperature of around 80° F. or above. Outbreaks of the disease occurred from July 5 to July 15 near Stockton, 1929, 1931, and 1932; near Colusa, 1931; and near Orland, 1932. Only a few cases of the disease were found near Stockton and Colusa in 1930 when the mean temperature was below 80° F. for most of the period shown on the graph.

In fact, maximum temperatures of 95 to 106° F. were recorded for every day of this period.

During the summer of 1930, repeated observations in the region where the blight had occurred in 1929, as well as in other regions of the interior valleys, were made and only a very few cases of the disease were found. Reference to figure 1 will show that no prolonged period of high temperature prevailed at Stockton nor at Colusa, in the Sacramento Valley, and that mean temperatures as high as 80° F. or above were rarely attained.

In 1931, when the blight was prevalent near Lodi, again, and also near Colusa in the Sacramento Valley, it occurred almost entirely in fields that had been planted just prior to or during the early days of an extended period of high mean temperature from June 30 to July 13. (Fig. 1.) The mean temperature was 80° F. or above during most of this time, and the maximum temperature at Colusa ranged from 96° to 108° F., and at Stockton was 100° F. or above on 8 of the 14 days. Many fields that had been planted earlier, in which the plants had attained some size before the extremely hot weather, showed no evidence of blight. The disease was severest where the plants had emerged from the soil during the period of high temperature. On July 17, 1931, an examination of approximately 500 acres of young Red Kidney beans on 8 farms near Colusa showed a loss of 5 to 50 per cent from seedling stem blight. One large planting of over 300 acres was damaged 5 to 10 per cent, and a 20-acre field, where the plants were just coming up during the severest part of the hot period, showed a loss of over 50 per cent. Several earlier plantings of Red Kidney beans where the plants had developed trifoliate leaves before the hot period did not show evidence of the disease.

In 1932 seedling blight was found very severe on July 13 near Orland, following a period of high temperatures extending from June 20 to July 9 (Fig. 1) during which the mean daily temperature was well above 80° F. on all but one day, was above 90° F. on 5 days, and reached 95° F. on June 30. The maximum temperature ranged from 101° to 113° F. for 17 of the 20 days and was above 95° F. the other 3 days.

Thus the occurrence of blight in the field was correlated with periods of hot weather when the mean daily temperature was 80° F. or above. During the 4 years 1929 to 1932, 3 such periods occurred and it was only during these periods that the blight seriously damaged bean seedlings. It is not unusual in the central valleys of California for periods of 1 to 3 weeks of hot weather to occur in June and July. It has been noted that beans planted so that the plants had developed the first trifoliate leaves before such a heat wave escaped the disease. Also, plantings made after the middle of July have not been observed to suffer materially from seedling blight.

The geographical distribution of this fungus as a pathogen suggests

that it is favored by high temperatures. Small (10, 11) reported that the wilt of beans caused by this fungus occurred at high temperatures and Taubenhaus (16) states that its attack on sweet potatoes is most common in overheated houses maintained at 80° to 90° F. during the sweating process and in storage bins next to or nearest the stoves.

SPECIES AND VARIETIES SUSCEPTIBLE

Under field conditions, Red Mexican, Red Kidney, and Pink beans, *Phaseolus vulgaris* L., have been seriously attacked, while not much damage has been observed in fields of Henderson's Bush Lima beans, *P. lunatus* L. var. *lunonanus* Bailey, or Blackeye cowpeas, *Vigna sinensis* Endl. In the 600-acre planting of Red Kidney beans, which showed a 50 to 60 per cent depletion in stand because of this disease in 1932, a small acreage of Blackeye cowpeas planted at the same time in one of the large bean fields showed no cases of the disease. Mackie (5) found the late-season attack of the fungus in California on Blackeye cowpeas and all varieties of beans except the Large Lima and Hopi Lima.

In a small experimental plot of infested soil at Davis, planted at intervals during the summer of 1932 with a number of species and varieties of

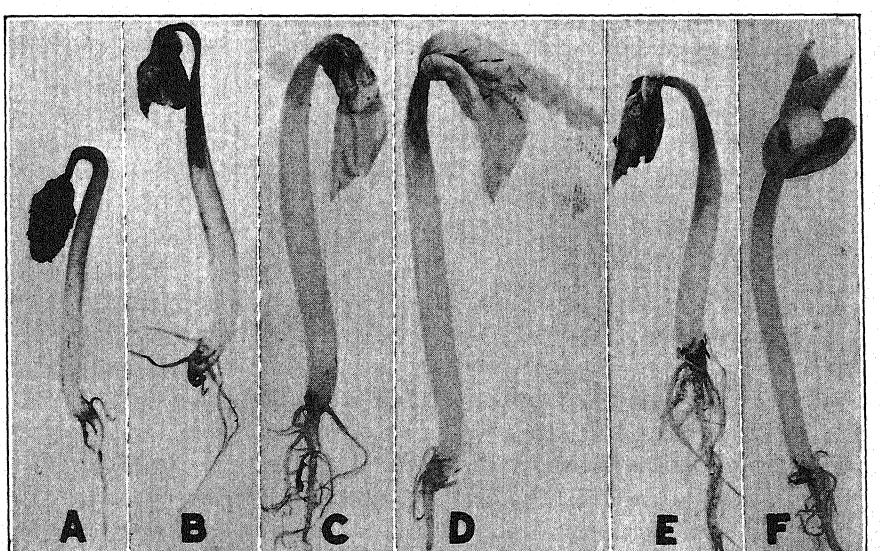


FIG. 2. Seedling Red Kidney beans showing different stages of stem blight caused by *R. bataticola* at high temperatures. A. Collapse of upper hypocotyl before the cotyledons reached the soil surface. B. Upper hypocotyl and plumule killed soon after emerging from the soil. C–E. Progressive stages in the development of stem blight on the hypocotyl and plumule after the plants have pushed through the soil. F. Healthy plant.

beans, light infection occurred on Scarlet Runner, *P. coccineus* L., Burpee's and Fordhook Bush Lima, *P. limensis* Macf. var. *limenanus* Bailey, and the following varieties of *P. vulgaris*: Speckled Cranberry, Davis White Wax, Kentucky Wonder, Golden Wax, Pink, Red Mexican, Dwarf Horticultural, Black Valentine, Refugee, Bountiful, and Stringless Greenpod.

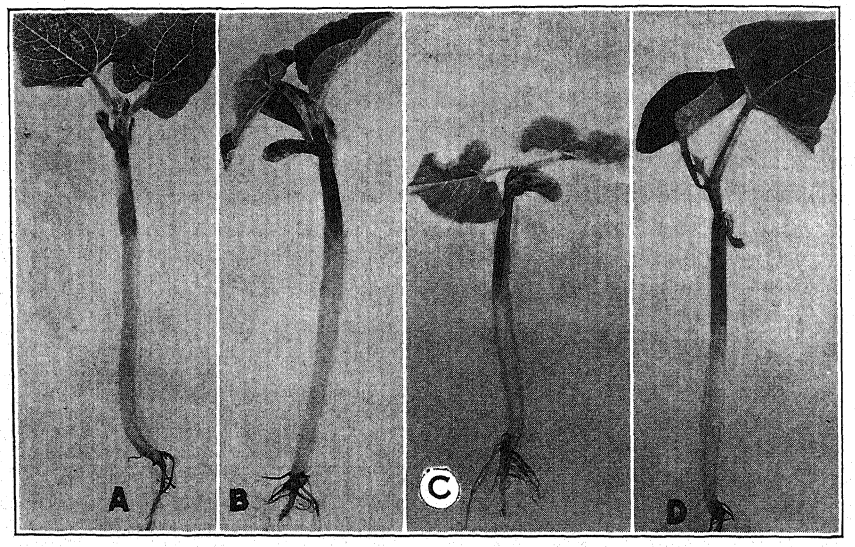


FIG. 3. Symptoms of stem blight on seedlings of Red Kidney bean after the primary leaves have developed. A. Incipient lesion at the cotyledonary node. B. Lesion approximately one day older than A. C. Linear lesion on one side of the hypocotyl and drooping of the primary leaves as a result of the invasion of the plumule by the fungus. D. Rapid invasion of the epicotyl and a primary leaf petiole, causing the petiole to collapse before the lamina wilts.

Seedlings Infected Near Cotyledonary Node. The fungus attacks the hypocotyl or epicotyl of the bean seedling either before or soon after it emerges from the soil, causing a conspicuous, black, sunken canker about the base of a cotyledon. (Fig. 3.) A somewhat similar type of hypocotyl infection was observed by Shaw (7) on jute, cotton, groundnut, and cowpea seedlings. Briton-Jones (2) obtained infection on cowpea and bean seedlings. Small (10) and Reichert and Hellinger (6) also obtained infection of bean seedlings. Small (13) found infection on seedlings of certain woody hosts, such as tea plants, and believed that infection occurred through the roots, though he (14) reports an instance of collar rot of a tea seedling. Butler (3) found that tea seedlings were invaded in the stem rather than through the roots.

The first evidence of the disease is the appearance of a small somewhat irregular, dark, sunken lesion on the stem at the base of a cotyledon petiole.

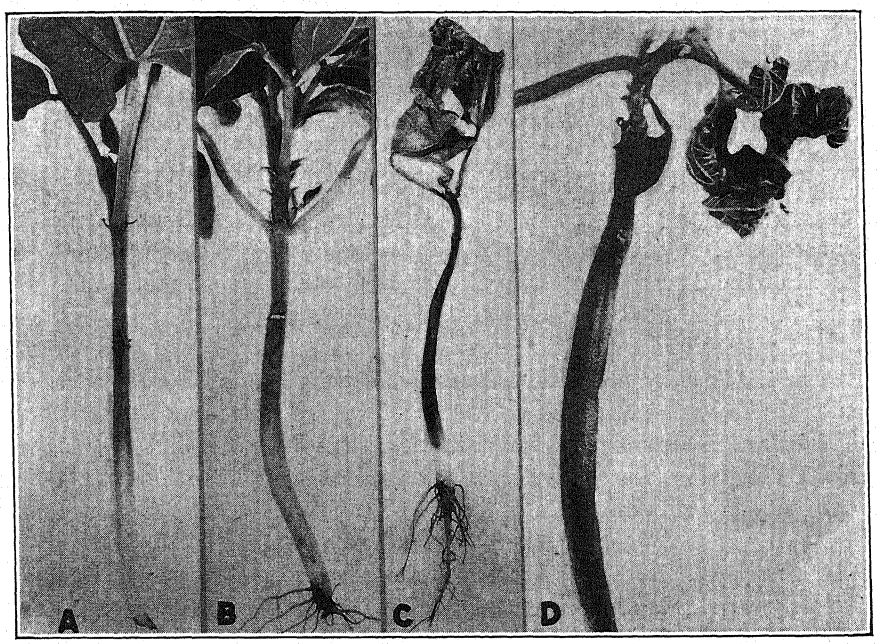


FIG. 4. A. Incipient lesion at cotyledon scar on a Pink bean plant where infection occurred after the trifoliate leaves started to develop. B. Late stage of the disease on an older Red Kidney bean plant showing the restricted development of the canker, concentric markings and a lateral break of the stem at the cotyledonary node. C. Advanced stage of stem blight of Red Kidney bean plant showing the death of the above-ground parts while the lower hypocotyl and roots are still apparently healthy. D. Red Mexican bean plant, killed by *R. bataticola*, showing small black, sclerotial bodies embedded in the cortical tissue.

(Fig. 3, A.) Infection spreads rapidly down the hypocotyl and up into the plumule, often involving the petiole of one of the primary leaves. (Fig. 3, D.) The progress of the lesion may be so rapid that the entire stem is involved, and the plumule is killed. (Figs. 2, A, B, and E and 4, C.) Often the petiole of a primary leaf is invaded so rapidly that the petiole tissues collapse before wilting of the lamina is evident. (Fig. 3, D.)

When very high temperatures prevail, the cotyledons and plumule are often invaded before the plant reaches the surface of the soil and when the U-shaped hypocotyl is pushed through the soil by the healthy roots below, the upper end of the hypocotyl, attached cotyledons, and plumule have been killed. (Fig. 2, A.) In other cases the plumule pushes out from between the cotyledons and starts to grow, but its development is quickly arrested by the progress of the fungus in the hypocotyl and epicotyl and the young plants are killed. (Fig. 2, B and E.) Many of these plants never reach the surface of the soil or, if they do break through the soil, they are in such

a weakened condition that they die and dry up in a few hours in the hot dry winds. Where irregularities in stand occur, it requires a rather careful examination of the surface of the soil, as well as just under the surface, to realize the extent of the damage to the young plants from this phase of the disease.

Often when infection is delayed until after the primary leaves are fully developed, the progress of the fungus is materially slower than when infection occurs before or soon after the plant emerges from the soil. The development of the disease on such plants is usually more pronounced along one side, resulting in the drooping and death of the primary leaf on that side and the yellowing of trifoliate leaves if present. Such plants are easily noted in the field by their stunted appearance and the yellow wilted condition of the leaves on one side.

If infection does not show until after the first trifoliate leaves are formed, the lesion is usually very much retarded in its development and is confined to the area immediately above and below the cotyledonary node. (Fig. 4, A and B.) Such lesions are sunken, dark-colored cankers with a rather sharp margin. It is not unusual to observe a concentric pattern in old slowly developing lesions. (Fig. 4, B.) Often the lesion extends down the stem to the ground line, but in no case has it been found extending to the roots. (Fig. 4, C.) However, even though the lesion is small, the stem becomes brittle at this point and usually is broken off sooner or later by wind or cultivation.

Sclerotial bodies are produced in the older parts of slowly developing lesions in 3 to 4 days, and in such lesions an abundance of small black bodies of this sort protrude from the cortex and are visible after 5 to 6 days. (Fig. 4, D.) Formation of sclerotial bodies is not so rapid on young plants infected while emerging from the soil, but after a few days these may be found in the dead tissues.

Macroscopic observation distinctly indicated that the tissue around the base of the small cotyledonary petiole was the point where the fungus had entered. Longitudinal and cross-sections, cut through the hypocotyl and attached cotyledon in both infected and healthy seedlings, failed to show any wound or natural opening in the tissues at the point of infection. Examination of young lesions showed that there was no invasion of the cotyledonary petiole until after the stem tissue at its base had been invaded. It, therefore, is assumed that the fungus is able to penetrate the unwounded epidermis, possibly through the area of primordial tissue in the axis of the cotyledon.

An examination of stained microtome sections through incipient lesions showed the fungus to be intercellular and confined in the early stages to the cortical tissues. The progress of the mycelium under favorable conditions

is rapid, causing a disintegration of the tissues. Growth of the mycelium is much more rapid longitudinally through the cortical tissues than radially or tangentially. Later the fungus penetrates the inner tissues and causes the stem to collapse.

RHIZOCTONIA BATATICOLA ISOLATED FROM DISEASED SEEDLINGS

Hundreds of isolations from diseased seedlings collected in the field and from artificially inoculated plants have consistently yielded pure cultures of *Rhizoctonia bataticola.* This fungus grows readily on potato-dextrose agar at ordinary laboratory temperatures. Mycelial growth is rapid, spreading, white, with slight aerial hyphae at first, but soon becomes more dense and prostrate. In 2 to 3 days at 25° to 30° C., the center of the mycelial mat begins to turn a greenish black, and at the same time, abundant, minute, black, round sclerotial bodies appear in the discolored area. Within 4 to 6 days the surface of an agar Petri dish usually is covered by the colony, and the small black sclerotial bodies, uniformly embedded in the upper strata of the medium, are so abundant that the fungus is black in appearance. In hundreds of agar plates and tube cultures examined, no pycnidia have been observed.

On sterilized young bean stems abundant growth occurs, and in a few days the small black sclerotial bodies appear in great abundance in the cortex. Such cultures have been examined from time to time over a period of more than one year during which they were allowed to dry thoroughly, but no pycnidia were found. Moreover, bean seeds were planted in sterilized moist sand in large test tubes with small bits of young agar cultures placed on the surface of the sand. The resulting seedling plants readily became infected, and in a few days the stems collapsed and numerous small black sclerotial bodies appeared in the cortical tissue. Microscopical examinations of these seedlings were made from the time infection showed up until the plants were dead and dry, but no pycnidia were found. Likewise, naturally infected plants from the field have been examined microscopically in all stages of the disease, but no pycnidia have been found. On inoculated sweet potatoes held in sterile moist chambers, typical charcoal rot, as described by Taubenhaus (15), was produced.

Apparently, this fungus is identical with *Sclerotium bataticola,* or *Rhizoctonia bataticola,* to which Ashby (1) applied the binomial *Macrophomina phaseoli* (Maubl.) Ashby. However, since the writer has found no pycnidia associated with this seedling disease, it seems advisable for the present to adhere to Briton-Jones' (2) and Haigh's (4) classification of the fungus as *Rhizoctonia bataticola* (Taub.) Butler. The size of the sclerotial bodies, which show an average mean diameter of 75.4 μ, indicates that the fungus apparently belongs to the small-sclerotia or C group of Haigh (4),

which includes his isolations from beans and in which he found pycnidia produced, and may very likely be identical with or closely related to the fungus causing ashy stem blight of beans[5,6] (17).

CULTURES PATHOGENIC IN SOIL AT HIGH TEMPERATURES

Infection of jute, cotton, groundnut, and cowpea seedlings grown in soil inoculated with cultures of *Rhizoctonia bataticola* (then termed *R. solani*) was obtained by Shaw (7), and infection of bean seedlings under similar conditions has been obtained by Small (10), Briton-Jones (2), and Reichert and Hellinger (6).

During the winters of 1929–30 and 1930–31 attempts were made to produce the disease on bean seedlings under partially controlled greenhouse conditions. In general, the method employed was to grow the fungus on sterilized oats in fruit jars and, after it had thoroughly permeated the oats, the contents were uniformly mixed with steam-sterilized soil, which was then placed in 6-inch pots. Beans were planted in the pots of soil. Although several hundred seedlings were grown under the above conditions during the two seasons, only a few slight cases of the disease developed. During the period of the above trials, the temperature ranged from 60° to 70° F., except for a few hours during the middle of the day, when the sunlight produced a somewhat higher temperature in the glass house.

Following the occurrence of the seedling stem blight again during and immediately after a period of abnormally high temperature in 1931, it appeared quite probable that high temperature played an important part in the occurrence of the disease. On July 22, 1931, cultures of *Rhizoctonia bataticola,* prepared as previously described, were uniformly mixed into steam-sterilized soil and 24 8-inch pots were filled approximately half-full of the inoculated soil. The 24 pots were divided into 2 groups and 12 to 15 beans of the Red Kidney variety were placed on the surface of the soil of each pot in one group of pots and a similar number of the Red Mexican variety in the other group. The seed was then covered with inoculated soil to the depth of 3 to 4 inches and the pots were placed in large galvanized pans on the south side of the greenhouse. A similar series of 12 pots was prepared from noninoculated soil for controls and placed in other pans near-by. Enough water was added to the pans at intervals to maintain the water level high enough for normal growth of the plants. When the pots were placed in the pans, thermometers were placed at 1-inch and 3-inch levels in the soil. Temperature readings were taken daily from these thermometers at 8 to 9 a. m., 12 to 1 p. m., and 4 to 5 p. m. There was no apparent difference in susceptibility between the Red Mexican and Red

[5] See footnote 4.

[6] See footnote 3.

Kidney varieties and they are not considered separately in the discussion to follow.

The number of plants in each series was recorded on August 10 and it was found that only 6 plants had emerged from the soil in the 24 pots of inoculated soil. By digging into the soil, it was found that the majority of the seeds had germinated, but had been killed before reaching the surface. The 6 plants emerging from the inoculated soil died shortly after coming through the ground and only one lived long enough to develop primary leaves. All showed unmistakable symptoms of stem blight and isolations yielded cultures of *R. bataticola*. There were 86 healthy plants in the 12 pots of noninoculated soil.

On August 10, seed of the same varieties of beans was again planted in all of the pots of inoculated soil and 6 pots of the control series. On August 20, 171 young plants were up in the 24 pots of inoculated soil and 37 showed stem-blight symptoms, while all of the 50 plants in the 6 pots of the control series were healthy. By August 22, 88 additional plants in the inoculated series had developed the disease, and by August 27 only 5 plants remained alive, 4 of which died within the next 4 days. A total of 201 out of 202 plants in the 24 pots of inoculated soil were killed by the disease (Table 1). All of the plants in the control series were vigorous and healthy on September 14, when the experiment was discontinued.

During the period July 22 to August 10 when the first test was being made, the mean air temperature, as recorded by the official weather station at Davis, ranged between 70° and 85° F. with an average mean of approximately 76° F. The average daytime soil temperature, determined by averaging the 3 readings of the triplicate thermometers at each depth, ranged between 95° and 113° F. at 1-inch depth and 81° to 95° F. at the 3-inch depth. (Fig. 5.)

For the period August 10–27, when the second series of inoculation tests were in operation, the mean air temperature ranged between 69° and 83° F. with an average mean of approximately 76° F. The average daytime soil temperature at the 1-inch depth ranged between 98° and 107° F., while at the 3-inch depth the variation was 82° to 89° F. (Fig. 5.) Thus, it will be seen that relatively high temperatures prevailed during these two series of inoculations in both of which approximately 100 per cent infection was secured.

In order to test further the effect of temperature on the occurrence of the disease, the same series of pots, which had not been disturbed, were again planted on September 14 with the same varieties of beans. Ten days after planting, 468 plants were up in the 24 pots of diseased soil and only 2 showed lesions. The experiment was continued until October 21 and a

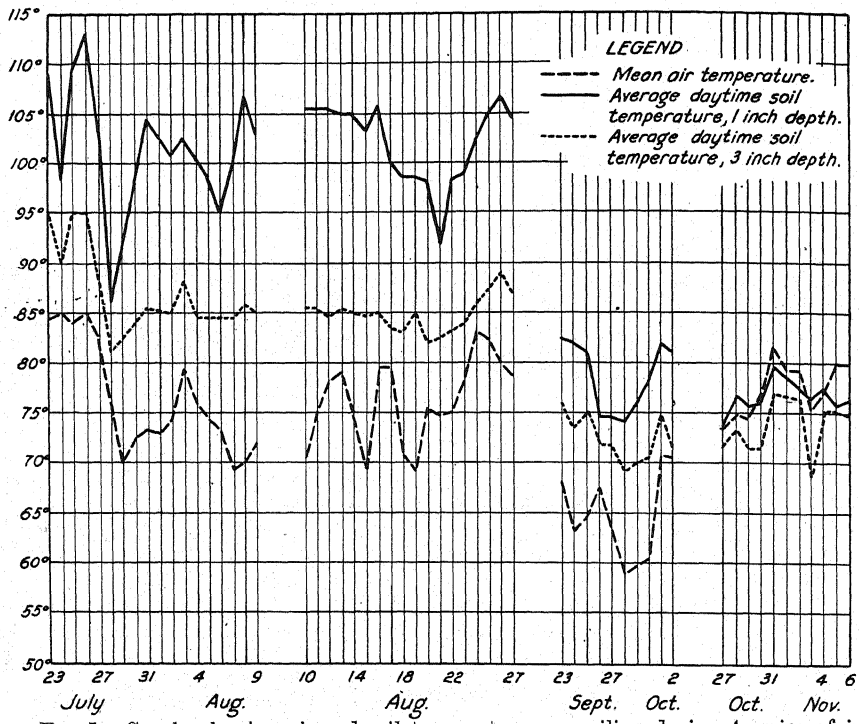


FIG. 5. Graphs showing air and soil temperatures prevailing during 4 series of inoculation tests with *R. bataticola* on bean plants, Davis, California, 1931. Abundant infection occurred during the first 2 series when high temperatures prevailed. Mean air temperatures taken from records of local office of U. S. Weather Bureau. Soil temperatures represent average of these readings at about 8 a. m., 12 m., and 4: 30 p. m., each day.

total of 37 of the 468 plants showed the disease, while 209 plants came up and grew normally in the noninoculated soil (Table 1). The progress of the disease during this experiment was very slow, only a few additional plants showing the disease from day to day. The lesions enlarged very slowly and very few of the plants were killed before the experiment was discontinued. During this experiment the mean air temperature was between 58° and 71° F., with an average mean of 65°; the soil temperature at a depth of 1 inch averaged between 74° and 82° F.; while the soil temperature at a depth of 3 inches averaged between 69° and 76°. (Fig. 5.) Thus, it will be seen that the temperatures were considerably lower than during the 2 earlier experiments.

As a further test, 12 of the pots of diseased soil and 12 pots of noninoculated soil were removed on October 21 to a greenhouse, placed in large pans on a bench, and seed was planted in the same way as in previous trials.

A total of 232 plants were produced in the pots of inoculated soil and only one plant showed the disease (Table 1). A very small canker developed at a cotyledon scar on this plant. There were 219 plants in the pots of noninoculated soil and all were healthy. The mean air temperature in the greenhouse during the time of the experiment ranged between 52° and 65° F., with an average mean of 61.2°; the soil temperature at a depth of 1 inch averaged between 72° and 80° F., and at a depth of 3 inches between 69° and 77° (Fig. 5).

TABLE 1.—*Results of inoculation tests at different temperatures in 1931. Seed beans were planted in pots of sterilized soil inoculated with a pure culture of R. bataticola*

Duration of test	Temperatures	Variety	Inoculum	Number of plants	Percentage diseased
	° F.				
Aug. 11 to Aug. 27	Air[a] 76.5	Red Mexican	*R. bataticola*	99	100
			none	25	0
	Soil[b] 98–107	Red Kidney	*R. bataticola*	103	99
			none	25	0
Sept. 23 to Oct. 2	Air 65	Red Mexican	*R. bataticola*	300	8.6
			none	125	0
	Soil 74–82	Red Kidney	*R. bataticola*	168	10.1
			none	84	0
Oct. 27 to Nov. 6	Air 61.2	Red Mexican	*R. bataticola*	141	0
			none	120	0
	Soil 72–80	Red Kidney	*R. bataticola*	91	1.1
			none	99	0

[a] Average mean daily temperature.
[b] Average daytime soil temperature at depth of one inch.

The effect of temperature on the growth of the fungus in pure culture was obtained by growing the fungus in Petri dishes of neutral potato-dextrose agar at the following temperatures: 39, 46, 54, 63, 68, 77, 86, and 104 degrees F. No growth took place at 39° and in one culture a trace of growth occurred at 46°, but no sclerotial bodies were formed. At 54°, growth and sclerotia formation were very slow and meager, while at 63°, fair growth took place and many sclerotia were formed in older cultures. There was good growth at 68° and 77°, the rate of growth and sclerotia formation being somewhat more rapid at 77°. Maximum growth and rapidity of sclerotia formation in these tests took place at 86°, while at 104° only a trace of growth occurred.

The results of these inoculation tests show that *Rhizoctonia bataticola* may cause a destructive stem blight of seedling beans and thus corroborate the theory suggested by the field observations, *i.e.*, that high temperatures are necessary for infection. Apparently the disease is favored by a daytime temperature of 95° F. or above in the surface inch of soil.

SUMMARY

Rhizoctonia bataticola causes a destructive stem blight of Red Mexican, Red Kidney, and Pink bean seedlings, which occurs in epiphytotic form in the central valleys of California. The disease may kill a considerable percentage of the seedlings and seriously decrease the stand.

Seedling stem blight is characterized by black, somewhat sunken lesions on the upper part of the hypocotyl, which may involve the epicotyl and plumule and promtly kill the plant. Plants not killed in the seedling stage usually are broken over later at the point where the canker has weakened the stem.

Infection takes place on the hypocotyl at the base of a cotyledonary petiole before or soon after the seedling emerges from the soil.

The fungus, *Rhizoctonia bataticola*, has been consistently isolated from diseased seedlings. No pycnidia have been found.

Epiphytotics occurred in 1929, 1931, and 1932 in fields where the seedlings came up during and immediately following periods of about 10 days or more in which the mean air temperature was 80° F. or more, with maximum daily temperatures near or above 100° F. There were no such periods in 1930 and no epiphytotics were noted.

Bean seedlings, grown in pots of sterilized inoculated soil, became infected in a manner typical of the disease as it occurred in the field during 2 series of tests in June and July, 1931, when the daytime soil temperatures averaged 95°–113° and 98°–107° at 1 inch and 81°–95° and 82°–89° F. at 3 inches, but not during 2 later series in September and October, when the daytime soil temperatures at a depth of 1 inch averaged 74°–82° and 72°–80° and at 3 inches, 69°–76° and 69°–77° F.

UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

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ISOLATION OF YELLOW-MOSAIC VIRUSES FROM PLANTS INFECTED WITH TOBACCO MOSAIC

JAMES H. JENSEN

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INTRODUCTION

The mosaic disease of tobacco, as is well known, is characterized by a blotching of the leaves with light and dark green areas. These areas produce the mottling or mosaic pattern, from which the disease derives its name. In addition to this mottling, small bright yellow spots occasionally occur on the leaves. They are few in number and may be easily overlooked. They are found, however, on most tobacco plants at some time in the course of systemic infection.

McKinney (4, 5, 6) has pointed out that the bright yellow areas contain a virus that differs from that of ordinary tobacco mosaic. He was able to isolate a yellow mosaic by cutting out the small yellow spots and inoculating from them. McKinney stated that the association of ordinary tobacco-mosaic virus and the yellow-mosaic virus might not constitute a contamination in the usual sense. He suggested that the yellow-mosaic virus might have arisen as the result of a mutation, although a satisfactory interpretation could not be given at that time because so few data were available. Dufrénoy (1) found that juice from bright yellow areas produced local yellow lesions when inoculated into mature leaves of tobacco.

Experiments were undertaken to determine whether the virus contained in the bright yellow spots is present in the original inoculum, occurs as the result of contamination, or arises in tissues invaded by the disease. The purpose of this paper is to describe the experiments and to report the results obtained.

MATERIALS AND METHODS

The tobacco-mosaic virus used in these experiments was of the ordinary field type and produced typical tobacco-mosaic symptoms.

The plants used in studies on bright yellow spots were *Nicotiana tabacum* L. variety Turkish, *N. sylvestris* Spegaz. & Comes, and *Lycopersicon esculentum* Mill. *N. glutinosa* L. was used in attempts to obtain samples of tobacco-mosaic virus free from all other viruses. *N. langsdorffii* Schrank was used in attempts to isolate pure samples of yellow-mosaic viruses.

All plants were grown in soil in 4-inch or 6-inch porous clay pots or in shallow wooden flats. Special precautions were taken to prevent accidental

[1] Published at the expense of the Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of acceptance of the manuscript.

infection of inoculated plants with other viruses. Touching the plants during watering and other greenhouse operations was carefully avoided. The greenhouses were fumigated frequently to control insects.

The following inoculation methods were used:

1. *Pot-label method.* Two small sterile wooden pot labels were used to break off and macerate a piece of diseased leaf. Transfers of virus to other plants were made by rubbing one or two small leaves with these pot labels. The advantage of this method is that it permits the easy transfer of several different viruses without contaminating the hands.

2. *Single pin-puncture method.* A sterilized ♯00 insect pin was inserted into that portion of a diseased leaf from which a transfer was to be made, and then into a small leaf (0.5 to 1.5 cm. long) of a healthy young tobacco plant. This method was used in transferring virus from bright yellow spots.

3. *Glass-spatula method.* A disk of diseased leaf tissue was cut out by means of a sterile cork borer and macerated on a glass slide with a glass spatula similar to that described by Samuel (7). Inoculations were made by rubbing leaves with the glass spatula. This method was used in making transfers from local necrotic lesions occurring on leaves of *Nicotiana glutinosa* and *N. langsdorffii.*

EXPERIMENTAL PROCEDURE

In preliminary experiments it was found that bright yellow spots from which a yellow-mosaic virus could be isolated occurred on leaves of *Nicotiana tabacum* variety Turkish, *N. sylvestris,* and *Lycopersicon esculentum,* whenever such plants were inoculated with tobacco-mosaic virus. The yellow-mosaic virus obtained from these yellow spots produced symptoms that differed from those of tobacco mosaic. Plants inoculated with mixtures of the virus of tobacco mosaic and that of the yellow mosaic developed symptoms that were different from those produced by either virus alone. When tobacco plants were inoculated with mixtures containing various proportions (1:1 to 1:100) of the 2 viruses, they were easily distinguished from those infected with only one virus.

It was found that the yellow-mosaic virus, rubbed over leaves of *Nicotiana glutinosa,* produced local necrotic lesions that appeared identical with those produced on this plant by tobacco-mosaic virus. When solutions containing mixtures of these 2 viruses were rubbed over leaves of *N. glutinosa,* there appeared necrotic lesions all of which looked alike. It was found, however, that when single necrotic lesions from such leaves were used to inoculate young tobacco plants, a high percentage of the plants produced symptoms characteristic of infection with one virus only. These results suggest that single necrotic lesions offer a means of separating the virus of tobacco mosaic from that of the yellow mosaic. The method was

used in attempts to obtain samples of tobacco-mosaic virus that would be free from the virus of yellow mosaic. These attempts were carried out in the following manner. Dilute solutions of the expressed juice of tobacco plants infected with tobacco mosaic were rubbed over leaves of *Nicotiana glutinosa* plants. A number of necrotic lesions were produced. Virus from isolated single lesions was transferred through a series of *N. glutinosa* plants, each being inoculated with virus from a single lesion of the preceding plant. Necrotic lesions on these plants were frequently tested for the presence of yellow-mosaic virus by inoculating young tobacco plants from single lesions. These plants always produced typical tobacco-mosaic symptoms. No bright yellow primary lesions, such as would have indicated that the virus of yellow mosaic was present, ever appeared. It is believed, therefore, that the virus of tobacco mosaic obtained from necrotic lesions was free from that of yellow mosaic.

With tobacco-mosaic virus obtained by this method, an experiment was next undertaken to determine whether yellow spots would occur on leaves of plants systemically infected and protected against contamination in the greenhouse.

Plants used in the experiment were tobacco, *Nicotiana sylvestris,* and tomato. Twenty healthy young plants of each species, which were later to be inoculated, were placed in the central portion of a greenhouse bench. Twenty similar plants, which were later to serve as controls, were placed so as to completely surround the test plants. This arrangement left the controls in the location where they would be most exposed to accidental infection, if any should occur.

All plants were kept under close observation for at least a week before being inoculated. Neither symptoms of tobacco mosaic nor chlorotic spots of any kind appeared on the plants during this preliminary observation period.

Inoculations on the twenty plants in the center of the group were made with virus from single necrotic lesions on *Nicotiana glutinosa* leaves. The tobacco and tomato plants were inoculated from lesions obtained on the 10th serial transfer, whereas plants of *N. sylvestris* were inoculated from lesions obtained on the 15th serial transfer. The glass-spatula method was used in making the inoculations. Of the 20 plants of each species inoculated, infection was obtained in 16 tobacco, 20 *N. sylvestris,* and 10 tomato plants. All of the 60 control plants remained healthy during the course of the experiments.

Within 10 days to 2 weeks after inoculation, some of the mottled leaves of the infected plants showed small bright yellow spots, similar to those observed in previous experiments. Other yellow spots continued to appear as long as the plants were held. Typical bright yellow spots are shown in

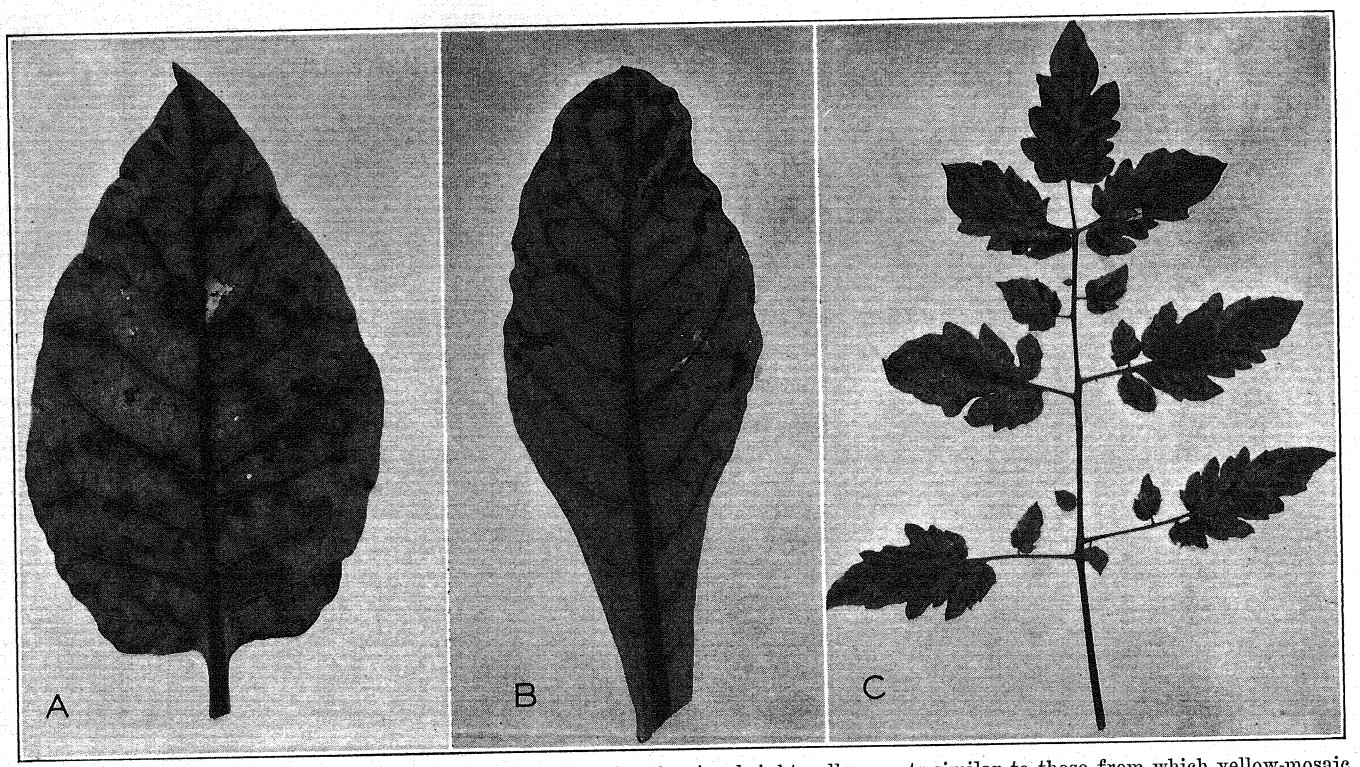


FIG. 1. Leaves of plants infected with tobacco mosaic, showing bright yellow spots similar to those from which yellow-mosaic viruses were obtained. A. Tobacco. B. *Nicotiana sylvestris*. C. Tomato.

figure 1. Of the 46 plants infected, all except 2 (1 of *Nicotiana sylvestris* and 1 of tomato) finally produced yellow spots. A total of 157 spots appeared during the period of 1 month following inoculation. Table 1 gives a summary of the results obtained. Since no chlorotic spots appeared on the 60 control plants in this experiment, it is concluded that the yellow spots on infected plants were not the result of contamination or of accidental infection.

TABLE 1.—*Summarized results of attempts to isolate virus from yellow spots in the leaves of Nicotiana spp. and tomato*

	N. tabacum	*N. sylvestris*	*L. esculentum*
Plants inoculated with tobacco-mosaic virus from necrotic lesions	20	20	20
Number of plants infected	16	20	10
Plants with yellow spots on leaves	16	19	9
Total number of yellow spots on infected plants	68	54	35
Isolations attempted	36	36	25
Yellow-mosaic viruses obtained	12	8	6

During the course of the studies, attempts were made to isolate virus from a number of the yellow spots. Leaves bearing yellow spots were removed from the plants by means of sterile scalpels. The single pin-puncture method was used in attempts to transfer virus from the yellow spots to young tobacco plants. Three to 5 days after inoculation, some of the small plants usually showed symptoms of infection. Six to 7 days after inoculation, the virus from each small diseased plant was transferred by the pot-label method to several healthy young Turkish tobacco plants. The symptoms on these plants were carefully observed. Plants showing infection with tobacco mosaic or with a mixture of tobacco mosaic and yellow mosaic were discarded. Those showing only symptoms of yellow mosaic were held separately. Twenty-six isolations of yellow-mosaic virus were made from yellow spots.

When the symptoms produced by inoculating plants from the yellow spots were studied, it became apparent that a number of different yellow-mosaic viruses had been obtained. It was observed that, while all plants inoculated with virus obtained from different yellow spots produced more or less similar primary yellow lesions, some produced distinctly different systemic symptoms. These observations suggested that the different yellow spots contained different viruses. Since these viruses were not the result of

contamination or accidental infection and, since their presence in the samples of tobacco-mosaic virus used for inoculation could not be demonstrated, even though repeated tests were made, it appears that they must have arisen in the infected plants.

For the purpose of comparing the viruses obtained from different yellow spots, attempts were made to inoculate various species of plants. Among the plants tested were the following: *Nicotiana glutinosa* L.; *N. glauca* R. Grah.; *N. rustica* L.; *N. sylvestris* Spegaz. & Comes; *N. paniculata* L.; *N. langsdorffi* Schrank; *N. acuminata* Hook.; *Lycopersicon esculentum* Mill.; *Physalis angulata* L.; *Solanum melongena* L. var. Hangchow Long.

Observations on the various kinds of plants inoculated with yellow-mosaic viruses showed that a number of the virus samples obtained from different yellow spots produced different symptoms on these plants as well as on tobacco.

On the basis of production of systemic symptoms in tobacco, the viruses appear to fall into 3 groups. Those in the first group produced general systemic infection with symptoms resembling tobacco mosaic but characterized by more intense yellowing. The viruses of this group all produced the clearing-of-veins symptom. This symptom was followed by the emergence of several leaves showing mottling and distortion. Such leaves were followed by 3 or 4 healthy-appearing, intensely green leaves. In many instances, these leaves showed no symptoms of disease until they were almost fully expanded, when blotch-like or ring-like yellow areas appeared. These yellow areas usually enlarged slowly until large portions of the leaves were involved. Later stages of the disease were characterized by the production of leaves that were more or less yellowed. The intensity of yellowing of the diseased leaf tissue varied with the different viruses of the group. Some viruses produced bright yellow chlorotic areas, others produced greenish yellow areas. The viruses producing the more intense yellow symptoms often affected the leaf tissue so severely that complete collapse and necrosis occurred. This was especially true of leaves involved in the clearing-of-veins symptom. Except for greater intensity of foliage symptoms the diseases in this group corresponded very closely with tobacco mosaic.

A second group of viruses produced systemic infections, which ordinarily advanced slowly and usually produced no typical clearing-of-veins symptoms. The first symptoms of systemic infection by viruses of this group consisted in the appearance of numerous small yellow spots on 1 or 2 of the young leaves. In some instances, these small spots tended to join together, forming a rough clearing-of-veins pattern; in other cases the yellow areas enlarged slowly until most of the leaf was yellowed. Symptoms appeared on leaves higher up on the stem in the form of blotch-like or

ring-like areas. Such spots, whether on the veins or elsewhere, enlarged slowly until most or all of the leaf was involved. The later stages of the disease were characterized by a slow enlargement of the existing yellow areas and the appearance of similar yellow areas on some of the younger leaves.

A third group contained a number of viruses that produced few or no symptoms other than the primary yellow lesions on the inoculated leaf. Some of them produced isolated yellow spots on 1 or 2 leaves above the inoculated leaf. Others produced chlorotic oak-leaf patterns, which appeared first on the leaves at the base of the stem and, later, on some of the leaves above. Several different virus samples were observed to produce no symptoms other than those on the inoculated leaf during a period of about a month following inoculation. The yellow lesions at the point of inoculation usually enlarged slowly until the entire leaf became yellow and finally collapsed. Several samples of the viruses produced yellow primary lesions that soon became necrotic.

The viruses of the 3 groups just described differ in the ease with which they are transmitted to healthy plants. Those of the first group are transferred readily, whereas those of the other 2 groups are transferred with considerable difficulty. Some of them are so difficult to transmit mechanically that even severe rubbing of the leaves with undiluted virus extracts does not give a high percentage of infection.

The yellow-mosaic viruses that have been isolated have not yet been sufficiently studied to justify a detailed description of symptoms. Brief descriptions of the most characteristic symptoms of 3 of them in tobacco and a few other plants are given below.

1. *Yellow mosaic, isolation No. 102.* This virus, which belongs in the first group, produces large yellow primary lesions on inoculated leaves of tobacco plants. These are followed by a sharply defined yellow vein-clearing and later by systemically infected leaves that are almost entirely yellow. A leaf of this type is shown in figure 2, A. Systemic symptoms of yellow mosaic are also caused by this virus on tomato, *Nicotiana sylvestris, N. glauca, N. paniculata,* and *S. melongena* var. Hangchow Long. Local necrotic lesions are produced on *N. glutinosa, N. acuminata,* and *N. langsdorffii.* Systemic necrosis is produced in *N. rustica* and *Physalis angulata.* The symptoms of the disease caused by this virus appear to be similar to those of Tobacco Mosaic No. 6 of Johnson (3), White Mosaic (2), and Aucuba Mosaic (8).

2. *Yellow mosaic, isolation No. 3.* This virus belongs in the third group. It produces large yellow primary lesions of indefinite outline when inoculated into tobacco leaves. These lesions continue to enlarge slowly, extending along the principal veins, as shown in figure 2, B. Usually, no symp-

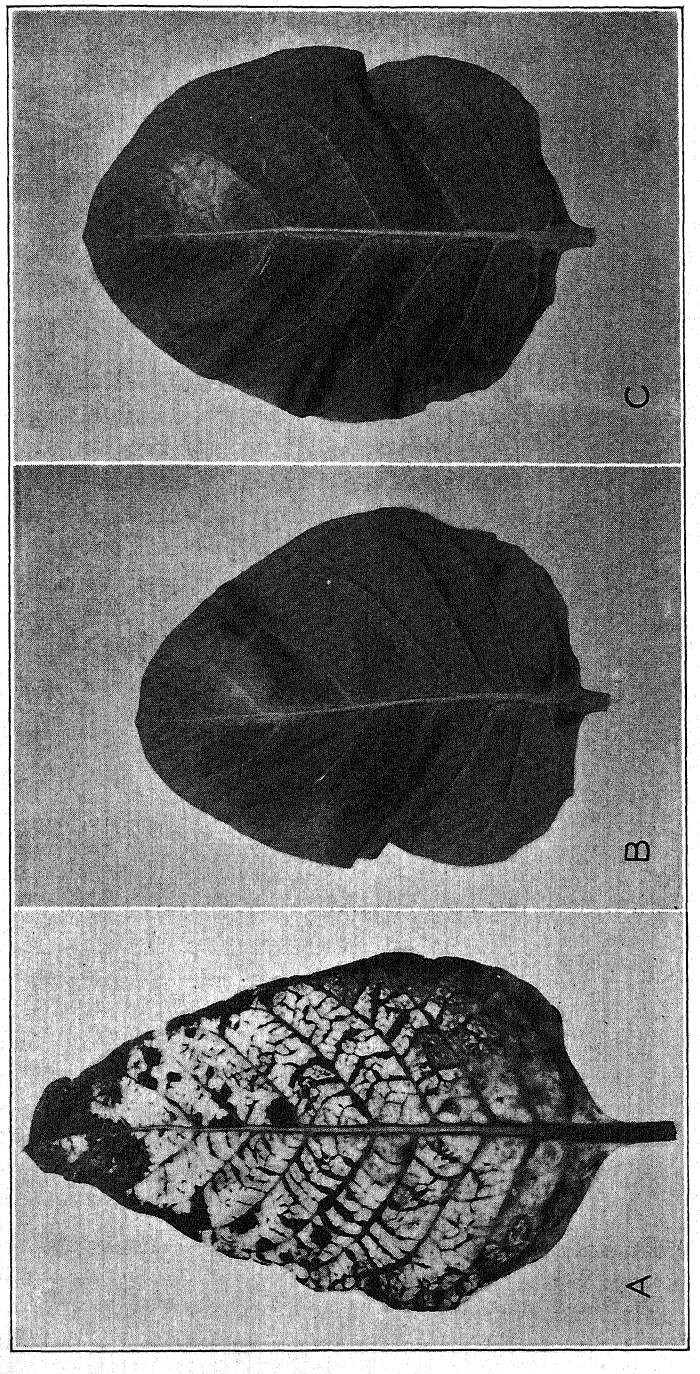


FIG. 2. Leaves of tobacco plants showing symptoms of yellow-mosaic viruses. A. Yellow mosaic, isolation No. 102, mottled leaf. B. Yellow spot, isolation No. 3, primary lesions. C. Necrotic spot, isolation No. 107, primary lesion.

toms other than the slowly enlarging spots appear on the plants. The spots finally extend over most of the affected leaf until, after several weeks, the entire leaf collapses. Large yellow spots, few in number, appear occasionally on 1 or 2 leaves above the inoculated leaf, but no vein-clearing or other symptoms of general systemic infection are to be observed. In one experiment, in which 15 Turkish tobacco plants infected with this virus were held about a month, symptoms appeared on leaves other than those inoculated in only 2 plants. This virus produces yellow blotch-like primary lesions on *Nicotiana sylvestris,* tobacco, and tomato; local necrotic lesions on *N. glutinosa* and *N. acuminata.* The virus was transferred to healthy plants with difficulty. So far as is known, the symptoms of the disease caused by this virus on tobacco have not been previously described.

3. *Yellow mosaic, isolation No. 107.* Primary lesions appear on tobacco leaves 4 to 5 days after inoculation with this virus, which also belongs in the third group. These lesions are of indefinite outline, grayish yellow, and soon break down to form blotch-like grayish brown necrotic areas (Fig. 2, C). Several weeks after inoculation, small brown necrotic spots and faint, slightly chlorotic, oak-leaf patterns appear on older leaves near the base of the inoculated plants. This virus produces local necrotic lesions on *Nicotiana sylvestris, N. glutinosa* and *N. acuminata.* It is difficult to transfer it to healthy plants. So far as is known, the disease resulting from infection by this virus has not been previously described. The difference between it and the viruses producing yellow spots is regularly shown when inoculations are made into similar plants in parallel experiments. It is possible that the virus would not produce necrosis on tobacco under all environmental conditions. In experiments conducted during the spring and summer months under varying light and temperature conditions, it invariably produced the symptoms described.

From these brief descriptions it is seen that the 3 viruses produce distinctive symptoms. Some of the other yellow-mosaic viruses produce less striking differences.

To test the possibility that the small differences might be due to mixtures, many of the viruses were reisolated from single necrotic lesions on leaves of *Nicotiana langsdorffii* in the following way. Each yellow mosaic was transferred to a separate set of *N. langsdorffii* plants. Six or 7 days after inoculation, 10 single necrotic lesions were cut out of the leaves of each set and used to inoculate 10 tobacco plants, each lesion being used on a different plant. In every instance, the 10 inoculated tobacco plants of a set gave identical symptoms. It was, therefore, concluded that the viruses thus tested were free from other viruses and that the differences observed resulted from differences inherent in each of them.

DISCUSSION

Evidence obtained during the course of this investigation indicates that yellow-mosaic viruses arise in bright yellow spots that regularly appear on the leaves of tobacco and tomato plants affected with tobacco mosaic. Some of these viruses cause diseases that differ markedly from each other and remain distinct in serial transfers. Others produce symptoms that differ so slightly that they are almost indistinguishable on the plants to which they have been transmitted. Still others cause diseases that seem identical.

It is believed that the possibility that these viruses may be carried along with the virus of tobacco mosaic in transfers from plant to plant or that they may be accidentally transmitted from some other plant to the tissues in which they appear, has been eliminated. Some of them are difficult to transmit to tobacco. Considering this characteristic, it seems improbable that they would be carried along in ordinary tobacco-mosaic inoculation. Since it is very difficult to recover them from the necrotic lesions they produce on *Nicotiana glutinosa,* it is even more improbable that they would be retained through a series of single necrotic-lesion transfers of tobacco mosaic such as was made in efforts to obtain pure samples of this virus. No bright yellow spots have ever been observed on uninoculated check plants. They occur only on plants that have tobacco mosaic and are far too numerous to permit their being considered as due to accidental contaminations. It is, therefore, believed that the yellow-mosaic viruses arise from the tobacco-mosaic virus. Although these viruses and the diseases they produce have not yet been sufficiently studied to justify conclusions regarding the extent to which they differ from each other or from the tobacco-mosaic virus, they are considered to be strains of the latter. All of them are readily distinguished from the latter by the symptoms produced in tobacco. Some also differ in showing a lower infectivity and a slower rate of spread from primary lesions. Insofar as has been been determined, they have the same host range as tobacco mosaic. In several different plants they produce necrotic primary lesions that are indistinguishable from those of the latter disease. Nothing is known as to the manner in which they arise, or the conditions favoring their production.

Some of the yellow mosaics of Solanaceous plants prevalent in commercial fields and greenhouses may have originated in yellow spots on leaves of plants having tobacco mosaic. It is possible that sucking insects that accidentally feed on the bright yellow spots on such leaves and later on healthy tobacco or tomato plants may thereby isolate yellow-mosaic viruses and start epidemics of such diseases as Tobacco Mosaic No. 6 of J. Johnson (3), the White Mosaic of E. M. Johnson (2), or Aucuba Mosaic (8). Some of the yellow mosaics isolated differ from any that have previously been described.

SUMMARY

Small, bright yellow spots, containing the viruses of yellow mosaics, occasionally appear on leaves of *Nicotiana tabacum* variety Turkish, *N. sylvestris,* and *Lycopersicon esculentum* infected with tobacco mosaic.

Similar yellow spots appear when plants of these species, protected against accidental infection, are inoculated with tobacco-mosaic virus shown to be free from all other viruses.

Twenty-six isolations of yellow-mosaic virus were made from yellow spots. Many of them differ from each other. The symptoms produced by 3 that showed marked differences are briefly described.

Evidence obtained in this investigation indicates that viruses of yellow mosaics arise during multiplication of tobacco-mosaic virus in infected plants.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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SOME SIGNIFICANT ESTIMATES OF LOSSES FROM PLANT DISEASES IN THE UNITED STATES

NEIL E. STEVENS

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Any one familiar with the literature of plant pathology in the United States will recall the frequency with which publications on the cause and control of a disease have included a statement of the importance of the disease, with an estimate, often expressed in dollars, of the losses which it was believed to cause. As is generally recognized, these estimates of crop losses were little more than guesses by informed and experienced observers, while the figures given in dollars were usually obtained by merely multiplying the commercial units estimated as lost by the sale price per ton or per bushel received for that portion of the crop actually sold.

WHY "DOLLAR" ESTIMATES ARE RARELY SIGNIFICANT

Obviously so simple a method of computing dollar losses can no longer pass even for purposes of propaganda. It is now clearly realized that not all losses from disease are of equal commercial significance. The economic losses from decay of fruits and vegetables in transit and on the market are much greater than the actual percentage of loss, for they involve the costs of harvesting, packing and shipping, as well as frequent claims for damage, and often what is more serious, lowering of the general price level and slowing-up of demand.

Losses from disease in the field, on the other hand, while often large and sometimes of tragic significance, usually involve only reduction in crop. This condition while serious enough to the individual grower may not appear so to the industry as a whole. Indeed, some pathologists[1] have gone so far as to suggest that "Viewed from the standpoint of the whole industry, the presence of these diseases is perhaps indirectly a benefit in its ultimate effect, since it serves as one of the natural checks on overproduction." Obviously, the difficulties in arriving at a fair understanding of the complex economic factors involved are too great to make possible even a reasonable approximation to dollar estimates of crop losses.

THE CROP LOSS ESTIMATES COMPILED BY THE PLANT DISEASE SURVEY

With the formal organization of the Plant Disease Survey in 1916 and under the stimulus of the demand for foodstuffs occasioned by war conditions an attempt was made to collect on a national basis estimates of losses

[1] Fawcett, H. S. and H. A. Lee. Citrus diseases and their control. 582 pp. McGraw-Hill Book Co., Inc., New York. 1926. (p. 1.)

due to disease in agricultural crops. The plan of operation, which has been continued to the present time, was to assemble from working pathologists throughout the United States, chiefly from the collaborators of the Plant Disease Survey, estimates in per cent of losses due to various diseases within their localities. The data obtained from all these sources were combined and preliminary estimates were sent to collaborators and to pathologists of the Bureau of Plant Industry for their final criticism and revision.

In these tabulations the losses were expressed in percentages of the total crop and in amount (in commercial units) of reduction in yield due to diseases. Reduction in yield was calculated by considering the production for the year as 100 per cent minus the percentage of loss from disease. The average estimated percentages of loss for the United States were obtained by dividing the total reduction due to all diseases by the sum of the known production plus the estimated reduction. This method was the one then followed by the Bureau of Crop Estimates. In spite of their admitted limitations, these estimates are probably the best that can be assembled for the period and will have decided historic interest as showing which diseases were considered most important by American students of plant diseases during this decade.

Finally, in 1931, a series of maps and charts was prepared by Miss Jessie I. Wood, which included the most complete reports of some of the

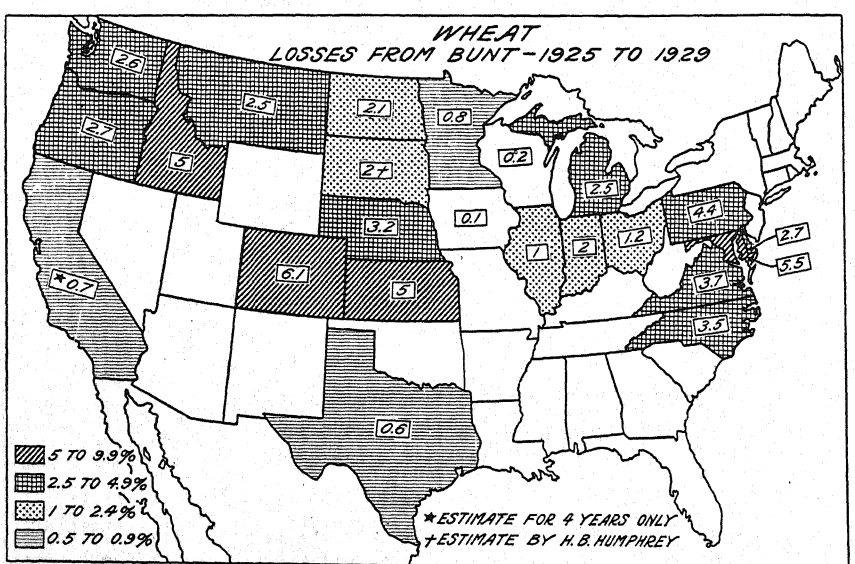


FIG. 1. Average estimated losses in wheat from bunt in various States. 1925 to 1929. States not shaded did not report regularly.

commoner and better-known diseases. These charts were distributed to pathologists for comments and suggestions. On the basis of these comments and our own study, 3 of these crop estimates have been selected that appear to be particularly significant or that can be checked by some other wholly independent method of estimating disease loss.

BUNT OF WHEAT

The estimates of losses from bunt of wheat for the 10-year period 1920–1929 are based on the reports from only 20 States and from this list some of the important wheat-producing areas are lacking. From 1925 to 1929 reports were, however, more numerous and as the reporting States are well scattered it seems probable that the figures are fairly representative. (Fig. 1.)

There is, moreover, supplemental evidence that the curve (Fig. 2) of

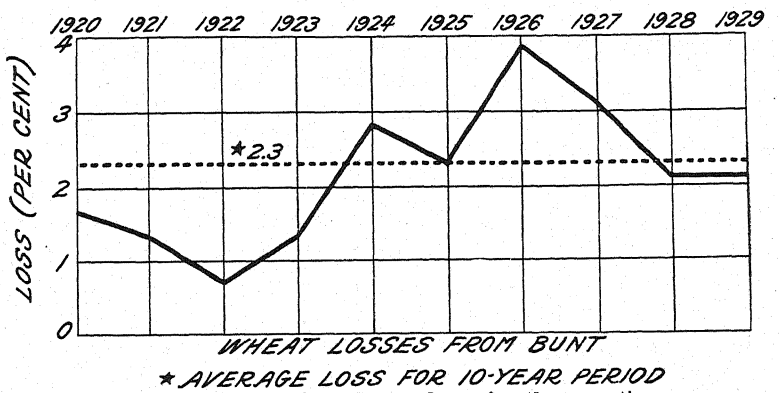


FIG. 2. Estimated losses in wheat due to bunt for the reporting area as a whole. 1920 to 1929. Average loss for the entire period is shown by the dotted line.

loss for the 10-year period is significant in its main features, that is, low losses up to and including 1923 and relatively high losses for 1924, 1926, and 1927, with somewhat lower figures for 1925, and since 1927. This evidence is found in the summary of information on "Smutty Wheat" compiled by E. G. Boerner, R. J. Haskell, and F. C. Meier. This summary consists of an analysis of the information on smutty wheat contained in the monthly reports of grain inspections for the United States as submitted by the various offices of Federal Grain Supervision of the Bureau of Agricultural Economics. The curve (Fig. 3) is made up of a simple average of the percentage of cars grading smutty at 15 important markets, from which reports are available for each of the years included. No doubt a weighted average that took into consideration the number of cars handled at each market would be more correct mathematically, but a simple average

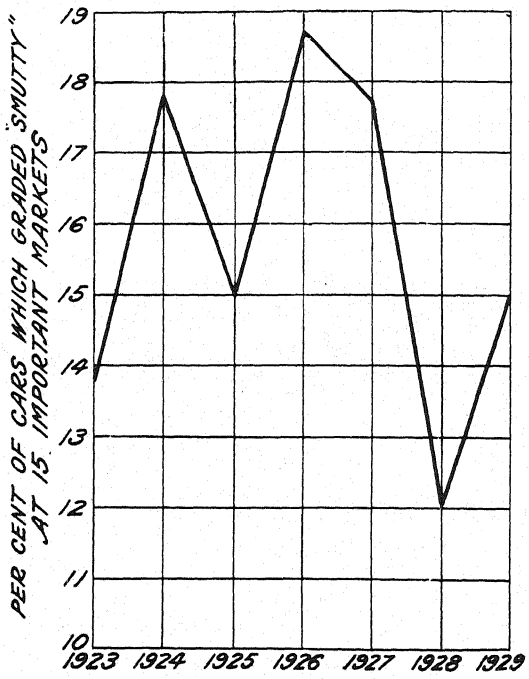


FIG. 3. Relative abundance of bunt in cars of wheat received at 15 important markets expressed as a simple average of percentage of cars which graded smutty. 1923 to 1929.

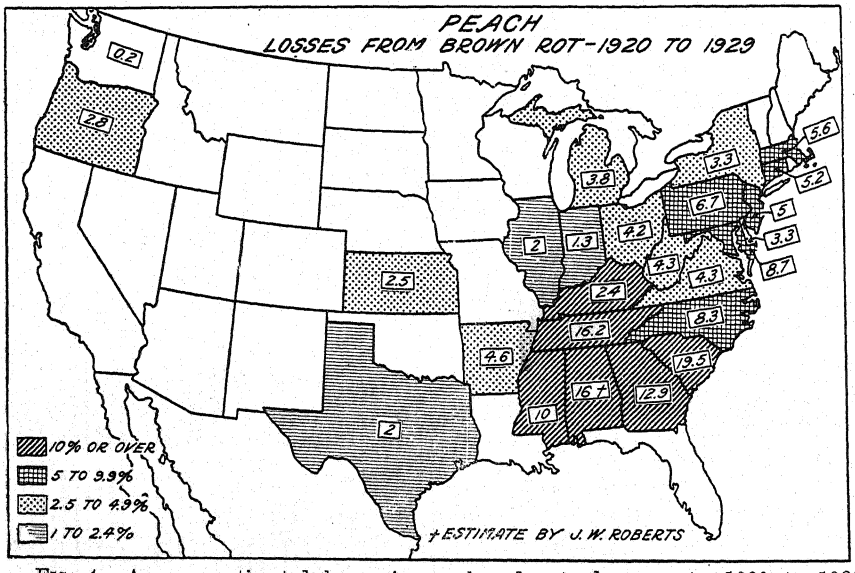


FIG. 4. Average estimated losses in peaches due to brown rot. 1920 to 1929. States not shaded did not report regularly.

shows as close a parallel as can be expected from figures derived from such widely different sources.

PEACH BROWN ROT

In the eastern part of the United States the losses from brown rot of peaches are, as is well known, much more serious in the Southern than in the Northern States. This appears on the map. (Fig. 4.)

The significance of the curves of estimated losses from peach brown rot (Fig. 5) is attested by a comparison with the curve based on the reports

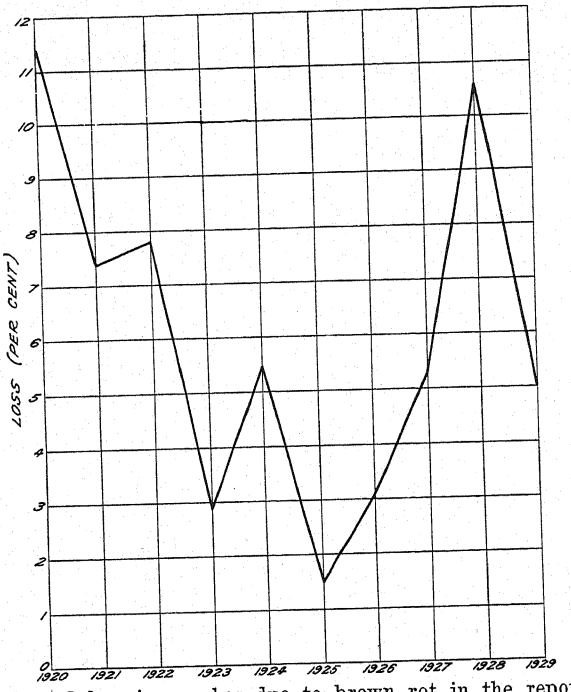


FIG. 5. Estimated loss in peaches due to brown rot in the reporting area as a whole. 1920 to 1929.

of the Food Products Inspection Service of the Bureau of Agricultural Economics. (Fig. 6.) In connection with a study of spoilage of stone fruits on the market, Brooks[2] analyzed these reports over the period of 1922–1928, inclusive, and published a careful tabulation of the results. These figures include the results of the inspection of over 7,000 cars of peaches. The actual number inspected in a single year varied from 752 in 1925 to 1,399 in 1928.

[2] Brooks, Charles. Spoilage of stone fruits on the market. U. S. Dept. Agr. Circ. 253. 1933.

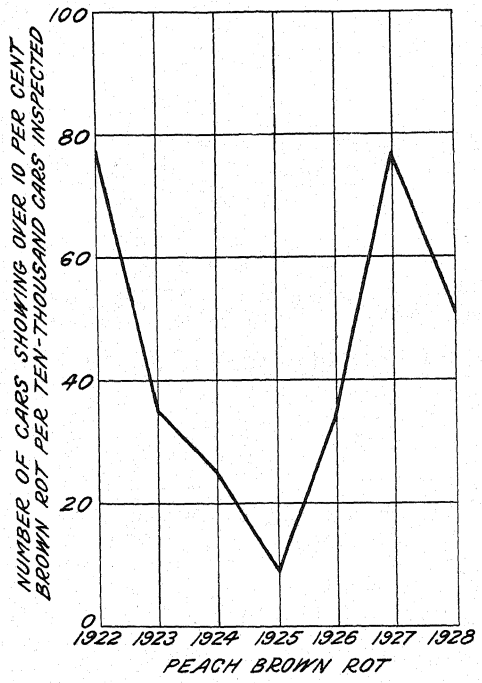


FIG. 6. Relative abundance of brown rot of peaches, 1922 to 1928, as shown by reports of the Food Products Inspection Service, expressed in the number of cars showing more than 10 per cent brown rot per 10,000 cars inspected.

The curve (Fig. 6) is based on Brooks' table and gives the percentage of cars inspected each year that showed over 10 per cent brown rot. The two curves thus deal with the same disease but are derived from wholly different sources. It must then be of significance that they show the same general trend—a sharp decline in losses from 1920 or 1922 to 1925, followed by a marked rise after that date.

CAUSES FOR THE FLUCTUATION IN BROWN ROT

Reference to the map shows the degree to which brown rot in the Southern States exceeds that farther north. Among these States, Georgia is preeminent in peach production; so great is this predominance that developments in this State overshadow all the others in the averages. J. W. Roberts' review of conditions in Georgia, which follows, appears to furnish a satisfactory explanation of the trend of the curves in figures 5 and 6.

Undoubtedly a considerable amount of the brown rot in 1920 was due to carelessness in spraying. In 1917 and 1918, in Georgia, there was almost no brown rot and no curculio. The impression became very general among growers that these pests had been practically eliminated. Spraying in 1919

and 1920 was of the most casual nature, and in 1920 both brown rot and curculio were very bad. In 1921, a serious spraying campaign was undertaken, the effects of which are plainly evident during the succeeding years, 1921 to 1926, inclusive. With the coming, however, of lower prices for peaches and the severe economic conditions of 1927 and the following years, there was a decided reduction in control activities and a corresponding increase in disease. (Unpublished memorandum.)

STORAGE ROTS OF SWEET POTATO

Losses to sweet potatoes from decay in storage have been the subject of careful study and have long been recognized as important. This was the only crop in which losses in storage were reported in sufficient detail to warrant compilation. The map (Fig. 7) is based on the reports of col-

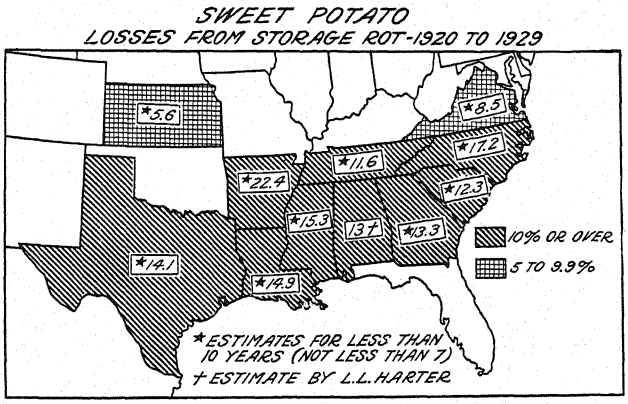


FIG. 7. Estimated losses to sweet potatoes due to storage rots for the reporting area as a whole. 1920 to 1929. States not shaded did not report regularly.

laborators, except for Alabama, which was interpolated on the advice of L. L. Harter.

The curve of storage losses (Fig. 8) apparently furnishes an example of the significance of extension work in plant pathology where effective control measures are known. Reduction of storage losses in sweet potatoes may be brought about by careful handling and drying of the potatoes after they are harvested, and the maintenance of proper temperature and humidity in the storage house. Storage losses also are materially reduced by the elimination of black rot and other diseases as a result of seed selection, seed treatment, and the application of other sanitary measures.

By 1920 the seriousness of the losses was fully recognized and the methods of control well known. An intensive campaign for their reduction, led by Dr. Harter, and no less effective for not being formally organized, was begun by Federal and State pathologists at this time. Early in 1922, F. C. Meier was made Extension Pathologist and took up the reduction

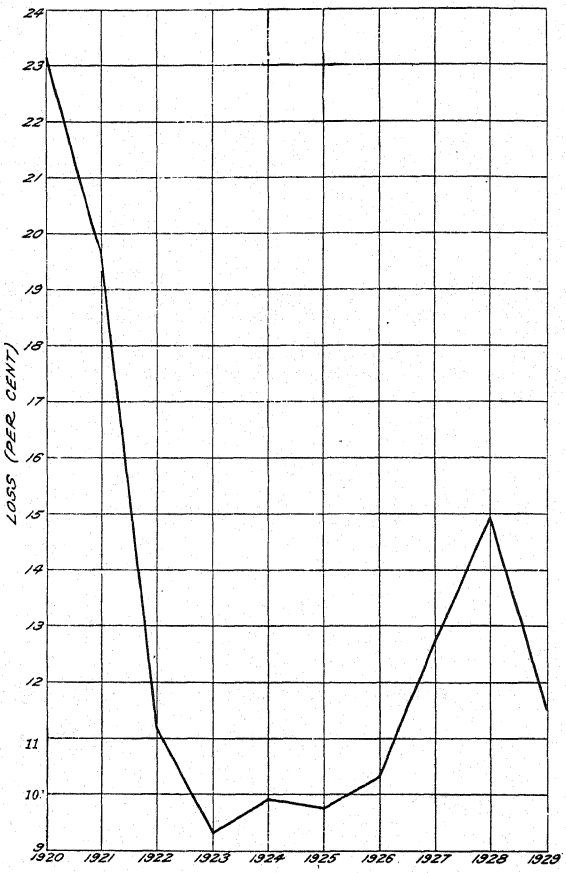


FIG. 8. Average estimated losses in sweet potatoes due to storage rots. 1920 to 1929.

of losses from rot in storage of sweet potatoes as one of his main projects. The result of these combined efforts was that in the 3-year period, 1920 to 1923, losses were reduced more than one half. The low level was maintained essentially unchanged for several years.

It should not be supposed that 1920, the first year shown on the chart, was characterized by unusually high losses from storage rot of sweet potatoes; in fact, losses estimated for that year are lower than for any of the few preceding years available. The average estimated losses for 1917, 1918, and 1919 were almost 28 per cent as against 23 per cent for 1920. In other words, the reduction shown on the chart indicates a new level as compared with that of the earlier period.

By 1926, however, Meier had diverted much of his attention to wheat-smut control, Harter had undertaken a study of bean diseases as his chief

project, and the low price of sweet potatoes had reduced the interest of the growers. A general increase in losses from storage rot followed.

ESTIMATES OF LOSSES FROM DECAY IN FOREST PRODUCTS

The emphasis here placed on estimates of losses from a few diseases that may be checked in more than one way should not be interpreted as meaning lack of interest in other types of estimates. Almost any sincere estimate by an informed observer has at least comparative value. Certain types of losses of great interest to pathologists are more easily measured than losses to growing crops. Examples of this are the losses by decay of fruits and vegetables in transit, which have been made the subject of several special studies, and even more conspicuously the losses from decay in certain forest products. Colley[3] has devoted to this subject a considerable amount of study, the results of which are largely unpublished, but certain of his estimates quoted below are typical.

TABLE 1.—*Estimated annual loss resulting from decay in forest products*

Estimated quantity of timber removed from forests of the United States		Estimated annual loss from decay	
Kind of material	Equivalent in standing timber in cubic feet	Equivalent in standing timber in cubic feet	Approximate per cent
Ties, sawed and hewed	1,320,000,000	660,000,000	50.0
Fence posts	1,800,000,000	900,000,000	50.0
Poles	55,250,000	27,625,000	50.0

Most of the other estimates in the same table are of almost equal significance but those given will serve as examples.

TOTAL YIELD AS AN INDICATION OF LOSS FROM DISEASE

Ordinarily so many factors other than disease, such as acreage and weather enter into total yields that it is not possible to relate yield directly to disease losses. There has recently been published, however, a report on cranberry production in New Jersey, which seems to be of direct significance in connection with the incidence of disease.

The smoothed curve of total cranberry yields (Fig. 9) in New Jersey prepared by Wilcox, but not published in his paper, indicates a fairly con-

[3] Colley, Reginald H. Utilization and conservation of the timber supply. Science, n. s. 61: 107–109. 1925.

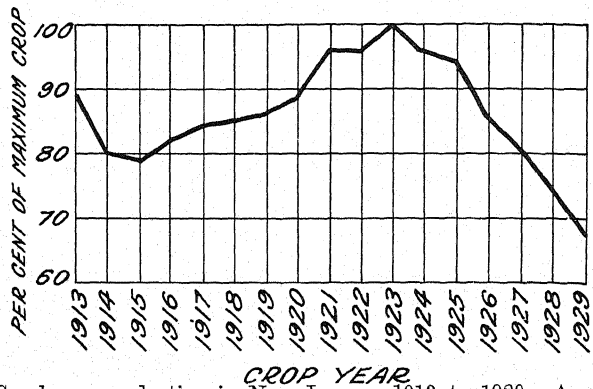


FIG. 9. Cranberry production in New Jersey. 1913 to 1929. A smoothed curve plotted in terms of the maximum crop of the period. The decline since 1923 is due almost entirely to the inroads of the false-blossom disease.

stant increase in yield from 1915 to 1923 followed by rapid and continued decline after that time. The significance of the production curve has been discussed by Wilcox,[4] and it is necessary here only to point out that the increase up to 1923 was largely due to the coming into bearing of newly planted areas, combined with the improved control of insects and fruit rots, and that the decline since 1923 has been chiefly if not solely due to the inroads of the false-blossom disease, particularly on comparatively recent plantings of Howes and older areas of native Jerseys.

The continued decline over a period of 8 years in the production of a standard fruit crop in the face of satisfactory, even increased, prices for the product furnishes perhaps the most striking possible evidence of the severity of this particular disease. To find a parallel it would be necessary to go back to the even more serious inroads of peach yellows in the Delaware Peninsula and the Blue Ridge regions in the last decade of the nineteenth century.

DIVISION OF MYCOLOGY AND DISEASE SURVEY,
BUREAU OF PLANT INDUSTRY, U. S. DEPT. AGR.,
WASHINGTON, D. C.

[4] Wilcox, R. B. Adjustments to false blossom in New Jersey. Proc. Amer. Cranberry Grow. Assoc. 63: 7–11, 14–15. 1932.

OBSERVATIONS ON TOLYPOSPORIUM FILIFERUM, CAUSE OF "LONG SMUT" OF SORGHUM[1]

M. N. KAMAT

(Accepted for publication March 8, 1933)

The long smut of sorghum (*Tolyposporium filiferum* Busse) was first described from East Africa in 1904 by Busse (3), who collected it from various parts of Tanganyika Territory, where it apparently did little damage. Barber (1) probably had this smut in mind when he described 3 smuts as occurring in the Madras Presidency, India, as the symptoms he

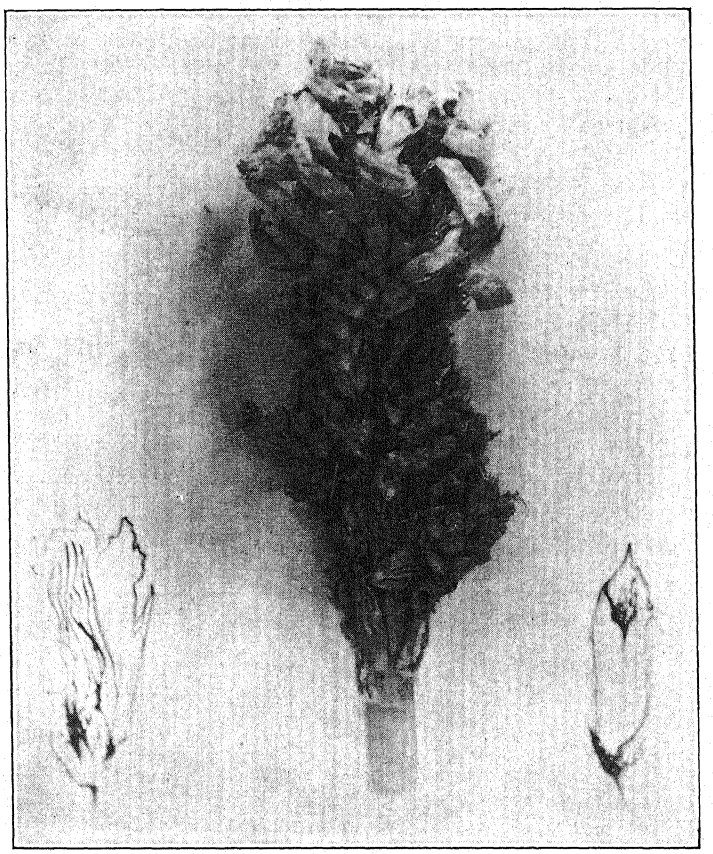


FIG. 1. Gross effects produced by *Tolyposporium filiferum* on sorghum. Note the long sori.

[1] The work reported in this paper was done in the laboratory of Plant Pathology, University of Minnesota. The writer is grateful to Dr. E. C. Stakman for the facilities and help afforded him during the investigation.

described coincide with those produced by this smut. Kulkarni (5) reports long smut from Bombay, India, and states that it is confined mostly to the extreme northern sections, particularly Sindh, but sometimes occurs in Kathiawar. He states that it causes heavy losses locally in certain years, although, in general, it is not considered a destructive disease.

Thomas (7) mentions the occurrence of long smut in Mesopotamia, and, according to Briton-Jones (2), it is common in Egypt. It has not yet been reported from the United States. Figure 1 shows the gross effect produced by this disease on sorghum.

Although long smut has been studied by Busse and Kulkarni, many essential points are lacking in our knowledge of the reaction of the pathogen to its environment. The investigation reported in the present paper includes a study of certain factors that influence the germination of chlamydospores, and it also includes a study of cultural characteristics of the of the organism on nutrient media.

SPORE GERMINATION

Kulkarni (5) obtained germination in water, dung solution, tomato broth, and glucose-peptone solution. Busse (3), on the other hand, reports germination only in the last solution. While Kulkarni (5) reports germination by both promycelium and germ tube, his figures illustrate only the former type. Busse (3) reported formation of long chains of aerial conidia

TABLE 1.—*The percentage and type of germination of chlamydospore clusters of Tolyposporium filiferum on several kinds of solid and liquid media at 28° C.*

Medium	No. of spore balls	Percentage of germination		
		By promycelium	By direct tube	Total
1 per cent potato-dextrose agar	81	14	82	96
Dung agar	78	12	53	65
Carrot agar	102	33	57	90
Nonnutrient agar	72	4	79	83
One per cent lactose	255	50	12	62
One per cent maltose	279	46	24	70
One per cent dextrose	276	48	13	61
Knop's solution	110	.....	.....	tr.
Sterilized distilled water	375	86	2	88

on the surface of a culture. Since extensive observations have not been recorded, the writer made a detailed study of germination.

Material for study was obtained from Bombay, India,[2] collected from the 1931 crop in Sindh. Germination tests were made both in Syracuse dishes and in hanging drops. The latter method was exclusively used for solid media. This was done by picking chlamydospore clusters from a dry coverslip and placing them on drops of various solid media with the help of a Zeiss micromanipulator. Observations and counts were made at the end of 48 hours. All the germination studies were made at 28° C. Several media were tried and the data obtained are tabulated in table 1.

The chlamydospores usually germinated readily on the solid and liquid media, and in sterilized water. All the chlamydospores in a spore ball frequently germinated but isolated individual chlamydospores germinated very capriciously. In the numerous trials made not more than 10 per cent of isolated single chlamydospores germinated.

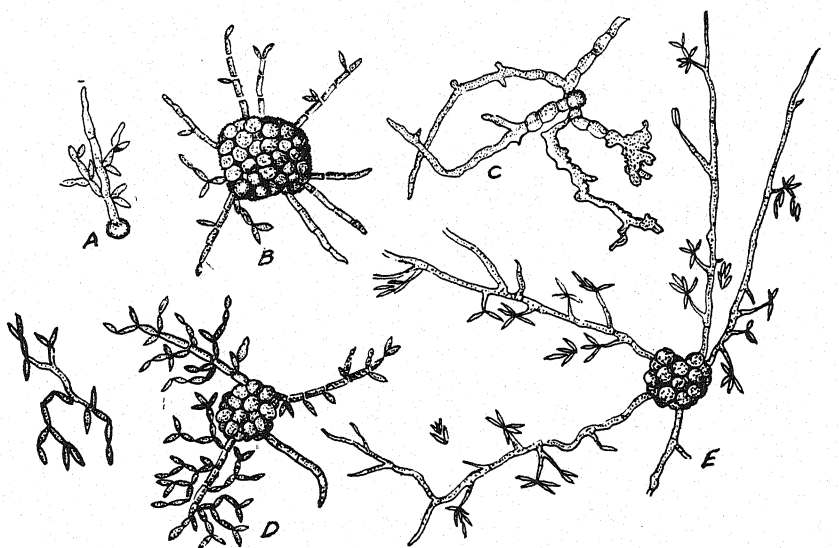


FIG. 2. Diagrams of various stages and types of germination of chlamydospores of *Tolyposporium filiferum* at 28° C. A, B, and D in sterile distilled water and C and E on 1 per cent potato-dextrose agar.

It is evident from table 1 that the percentage of germination on dung agar was much lower than on the other solid media, possibly because of its higher acidity. Germination was much more vigorous, as measured by the length of tubes, on 1 per cent potato-dextrose agar, carrot agar, and on the nonnutrient agar. As a rule, typical promycelia and sporidia were formed

[2] The writer is grateful to Dr. B. N. Uppal, Plant Pathologist, Agricultural College, Poona, India, for the supply of chlamydospores.

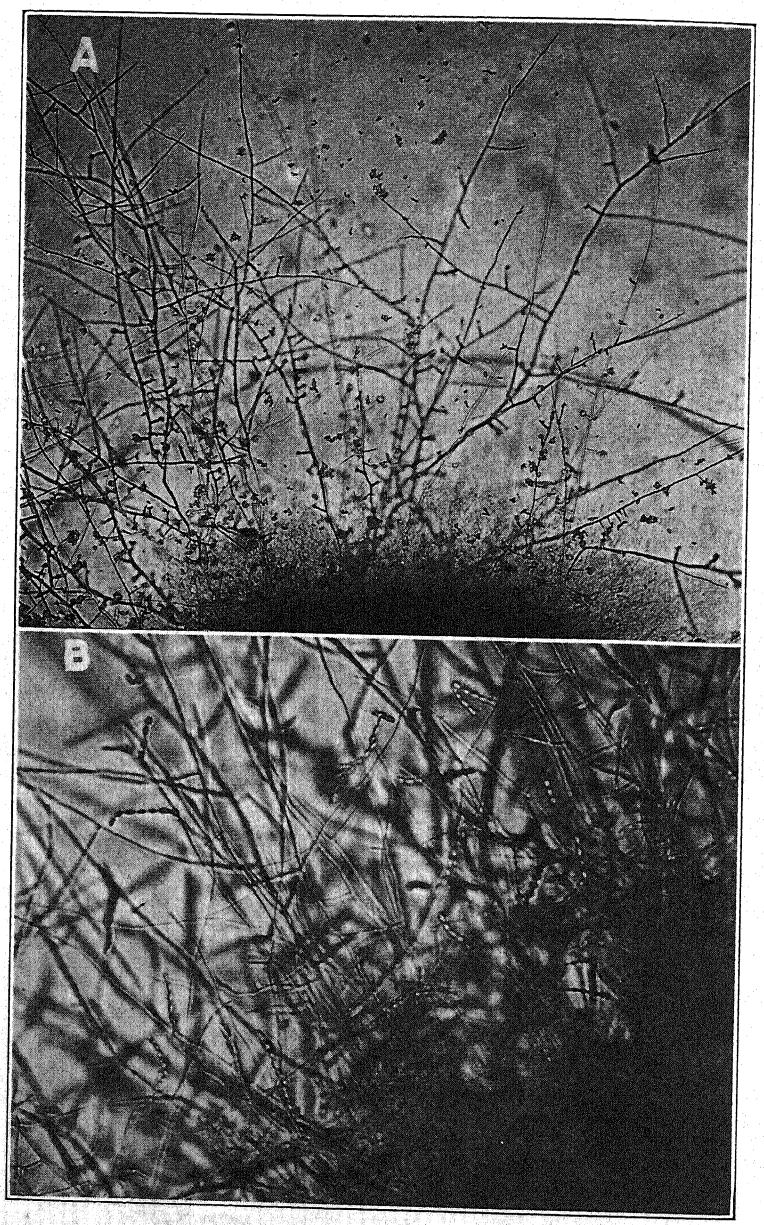


FIG. 3. Two types of germination in *Tolyposporium filiferum*. A. Aerial conidia borne in clusters. B. Aerial conidia borne in chains.

most often in liquid media and especially in sterilized distilled water (Fig. 2, B and D), while on solid media the chlamydospores commonly produced germ tubes, which branched laterally, forming aerial conidia, either in long chains (Fig. 3, B) or in clusters at ends of short, pointed, thorn-like branches (Figs. 2, E; 3, A). The former method of conidial formation apparently was favored by a relatively dry environment, while the latter type was induced by a moist medium.

Striking variations were noticed in the promycelial type of germination. The promycelium was generally quadricellular, but sometimes tricellular, and, in some cases, promycelia of 5 and 6 cells were observed. The sporidia were spindle-shape and from 8 to 24 μ long. They were formed both laterally and terminally, often in clusters at single points and on both sides of the promycelium; in some cases, they were formed directly from the chlamydospore, without the formation of a promycelium. The aerial conidia were much shorter and measured from 4 to 8 μ in length.

Studies were made to determine the cardinal temperatures for the germination of chlamydospores. One per cent potato-dextrose agar and sterilized distilled water were used for these tests. The results are summarized in table 2.

The data indicate that optimum temperature for germination of chlamydospores is about 28° C. However, the percentage of germination at 33° C. was almost as high as at 28° C. The minimum was between 10 and 13° C., while the maximum was a little above 39° C.

TABLE 2.—*Percentage of germination of chlamydospores of Tolyposporium filiferum in one per cent potato-dextrose agar and in sterilized distilled water at different temperatures*

Temperature in °C.	One per cent potato-dextrose agar		Sterilized distilled water	
	No. of spores	Percentage germination	No. of spores	Percentage germination
10	104	0	85	0
13	60	50	0	.....
15	70	52	92	33
20	180	59	102	69
25	82	83	148	73
28	108	91	105	82
33	120	88	98	78
36	80	31	80	19
39	75	8	65	tr.

CULTURAL CHARACTERS

Approximately 100 monosporidial lines of *Tolyposporium filiferum* were isolated and studied to some extent. The method of isolation was essentially that used by Hanna (4). The sporidia were numbered 1, 2, 3, and 4 in order from the tip of the promycelium to chlamydospore. Sometimes it was difficult definitely to locate the position, because of the clusters of sporidia produced at each point, in which case, letters a, b, c, etc., were used for designating such lines. A number also was assigned to an individual chlamydospore whenever a culture was made from it. Thus 16–1 refers to a line that originated from the tip cell of the promycelium of chlamydospore No. 16.

Several media, such as carrot agar, carrot agar + 1 per cent dextrose, nonnutrient agar, dung agar, and Brown's artificial potato agar were tried in addition to 1 per cent potato-dextrose agar. None of them promoted good growth except the last one. On carrot agar the growth was slow and the diameter of the colony small. On carrot agar to which 1 per cent dextrose was added the growth was still meager. On nonnutrient agar, the growth was very slow, flat radial, silken in texture, and white myceloid.

Figure 4 shows the comparative growth of line 16–2 in 1 per cent potato-dextrose agar and in carrot agar under standard conditions. There was a marked tendency for sectoring on carrot agar. These wedge-shape sectors were usually smooth, mycelioid, and so membranous in consistency that it

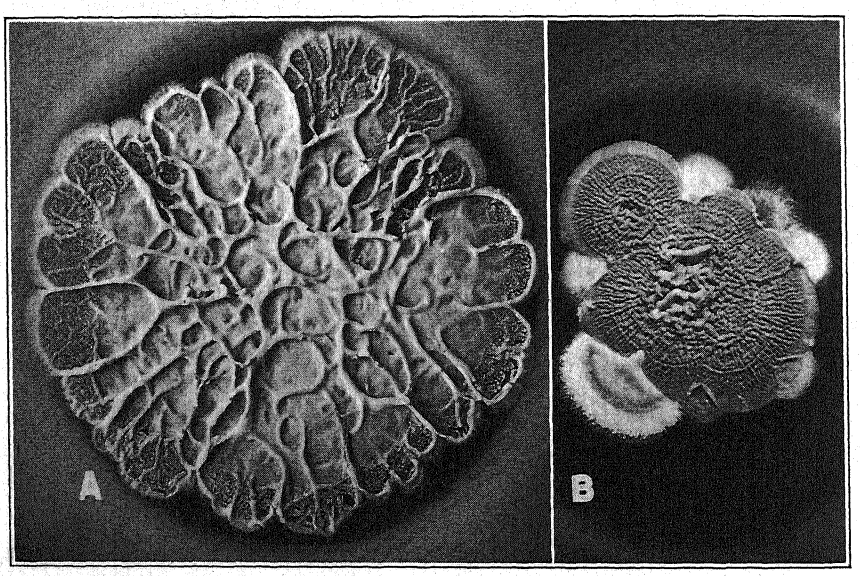


FIG. 4. Growth of monosporidial line 16–2 at end of 10 days. A. On 1 per cent potato-dextrose agar. B. On carrot agar. Note sectors.

was difficult to make transfers from such sectors except by cutting pieces with a sharp spatula.

Most of the monosporidial lines isolated showed no marked differences in their cultural characters when grown on several different kinds of media. A few, however, were distinctly different. Four of these lines and one monochlamydospore culture were selected for further study.

The cultural characteristics of 5 lines of smut on 1 per cent potato-dextrose agar are given in table 3. The apparent differences were chiefly

TABLE 3.—*Cultural characteristics of five lines of Tolyposporium filiferum grown on 1 per cent potato-dextrose agar for 9 days at 28° C.*

Line	Color[a]	Topography	Surface	Margin	Consistency	Diameter of colony in mm.
19	Pale cinnamon pink	Raised Broadly ridged	Dull	Lobate	Membranous	40.0
16–2	Buff	Raised Finely ridged	Dull	Wavy	Membranous	50.0
11–1	Pale cinnamon pink	Rugose Broadly ridged	Dull	Lobate	Leathery	50.0
20–2	Pale cinnamon pink	Raised Broadly ridged	Waxy	Lobate	Yeastoid	37.5
13–d	Ivory yellow	Raised Broadly and deeply furrowed	Waxy	Broadly lobate	Yeastoid	40.0

[a] According to color standards and nomenclature of Ridgway (6).

in color, consistency, margin, and topography of colonies. Certain lines also could be distinguished by their rate of growth at different temperatures.

The effect of temperature on the rate and type of growth of the 5 cultures on 1 per cent potato-dextrose agar was determined. Flasks of 250 cc. capacity, with 40 cc. of medium, were inoculated in duplicate and were incubated in the dark and kept at different temperatures. At higher temperatures, as a general rule, the colonies tended to be membranous in consistency, much paler in color, with less distinct and more regular margin, and the ridges were finer than those at lower temperatures. At lower temperatures, namely, between 20° C. and 25° C., the cultures were brighter in color, raised, and deeply and broadly ridged in topography; the margin was broadly lobed, and the consistency yeast-like.

Two monosporidial lines, 16–2 and 13–d, showed very marked differences in their rate of growth; line 16–2 grew much more rapidly than did line 13–d. This difference was maintained at all the temperatures in all the

trials made. The temperature range for growth of line 13–d was 13° C. to 36° C.; it was 10° C. to 39° + C. for line 16–2. The optimum temperature for line 13–d lies between 28° C. and 33° C., while that for line 16–2 is between 33° C. and 36° C.

It would appear that the optimum temperature for growth in culture is not the same as for chlamydospore germination. Lines 13–d, 19, and 20–2 showed very close agreement between the germination tests made on potato-dextrose agar, while 16–2 and 11–1 had higher optimums as well as wider growth ranges. No definite cardinal temperatures, therefore, could be determined, since there was so wide a variation between lines. In general, however, it can be said that the optimum for growth in culture lies between 28° C. and 36° C.

SUMMARY

Tolyposporium filiferum, causing long smut of sorghum, is sometimes destructive in certain parts of India, but it has not been reported from the United States.

Chlamydospores of long smut germinate readily on many different types of medium and over a wide temperature range. In water and in liquid nutrients typical promycelia are usually developed, while on solid media the chlamydospores commonly produce a germ-tube-like growth, which branches and forms aerial conidia either in long chains or clusters.

Approximately 100 monosporidial lines were isolated and studied culturally on solid nutrient media. Most of these smut lines were alike; a few, however, differed strikingly from each other in the following cultural characteristics: color, consistency, margin, surface, topography, and rate of growth of the colonies. Wedge-shape sectors were frequently observed in monosporidial lines when grown on certain nutrient media.

The influence of temperature on growth in culture varied with the different lines, but the optimum temperature for growth of all lines studied lies between 28° and 36° C.

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BOTRYTIS CINEREA AS A CAUSE OF POTATO TUBER ROT

DONALD FOLSOM

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Pethybridge (14, pp. 577–578), in 1916, while discussing the *Botrytis cinerea* blight of potato vines in Ireland, stated that this fungus does not affect the tubers. However, it is of interest that, from tubers affected with a "watery" or "glassy" rot, he sometimes isolated "pure cultures of a Botrytis which appeared to be identical with *B. cinerea*" (14, p. 591). He states that "this form of rot . . . has only been found to occur in . . . tubers which . . . remain attached to the parent plant at the time of digging," and "no infection experiments were made with the Botrytis, as previous trials with the same fungus isolated from leaves gave negative results" (14, p. 591).

The apparent immunity of the tubers is again pointed out by Cotton in 1919 (9, p. 43). Brown (8, p. 291), in 1922, related that potato-tuber tissue gives off volatile substances that retard the germination of spores of *Botrytis cinerea*. He concluded that the atmosphere of a potato storage place is very unfavorable to Botrytis germination (8, p. 298) but points out that "as regards the effect of volatile constituents of the plant on the growth of the fungus, once the latter is inside, little can be said at present" (8, p. 298).

The isolation of a Botrytis in pure culture from several soft-rotted tubers stored in Maine was reported in 1927.[1,2]

The writer in the spring of 1932, while examining potato tubers (*Solanum tuberosum* L.), of the Green Mountain variety, stored in a typical potato cellar in northeastern Maine, found tubers with apparently a new kind of rot. The exterior of the tuber showed a flabbiness and a wrinkling of the skin that were more pronounced as one approached the stem end of the tuber. (Fig. 1, A and B.) A cut through the conspicuous junction of flabby and normal solid tissue disclosed a rot that was rather soft, though not wet unless squeezed, and that was advancing in the interior and causing a temporary darkening of the flesh. (Fig. 1, B.) Where the rot had penetrated the tissues, the eyes were filled with tufts of gray mold (Figs. 1, A, B, and 2, A) with which blue mold (Penicillium) was sometimes associated. From the "frontier" of the advancing rot, pure cultures of a Botrytis were readily isolated. With these cultures and others

[1] U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rprtr. 11: 54. (Mimeographed.) 1927.

[2] Diseases of vegetable and field crops (other than cereals) in the United States in 1927. U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rprtr. Sup. 61: 237. (Mimeographed.) 1928.

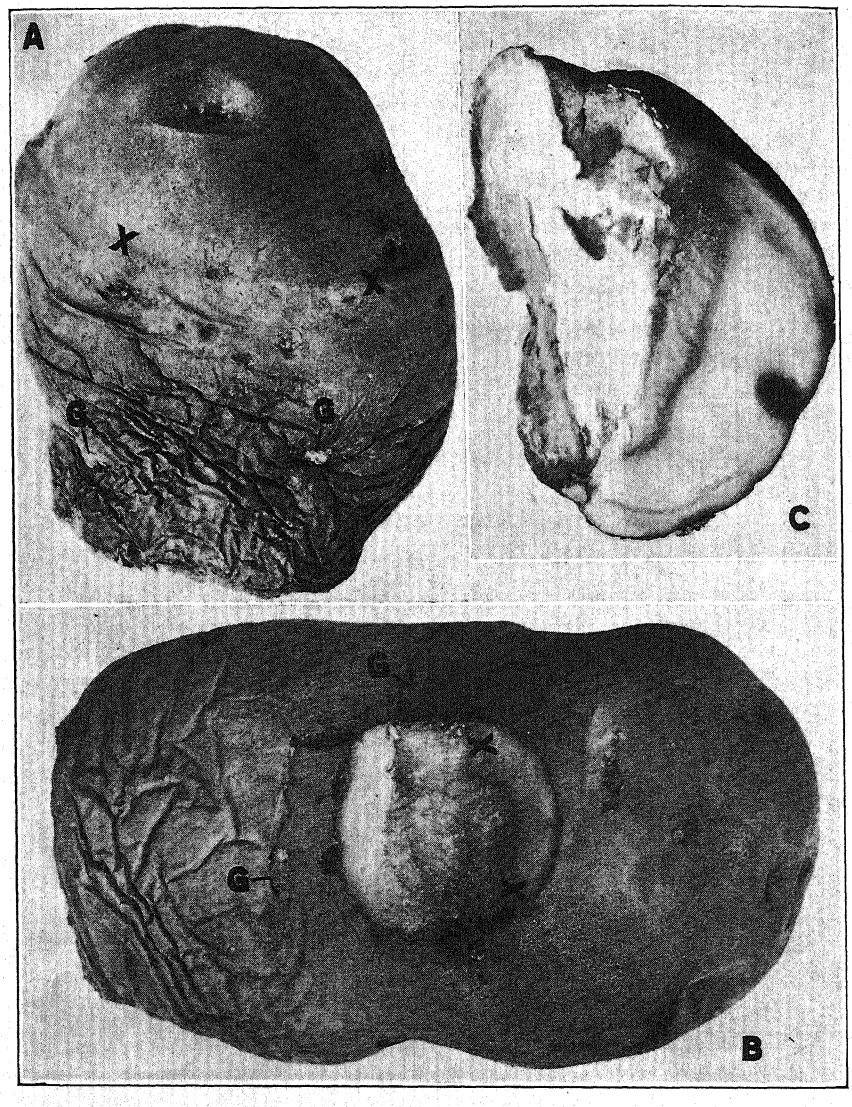


FIG. 1. Green Mountain potatoes. A. Tuber from storage. *Botrytis cinerea* had penetrated through tissues to X–X and emerged (G) from eyes. B. Like A except for cut. C. Half tuber like B except inoculated with pure culture. Reisolation effected from this lesion.

of the same fungus, in due time, tuber infection (Figs. 1, C and 2, C) was obtained identical with that found in storage in 1932, as well as a more shallow, arrested kind of lesion (Fig. 2, B), with most of the surface Pinkish Buff by Ridgway (15, Pl. XXIX) or of color F-4, Pl. 10, in Maerz and Paul (11).

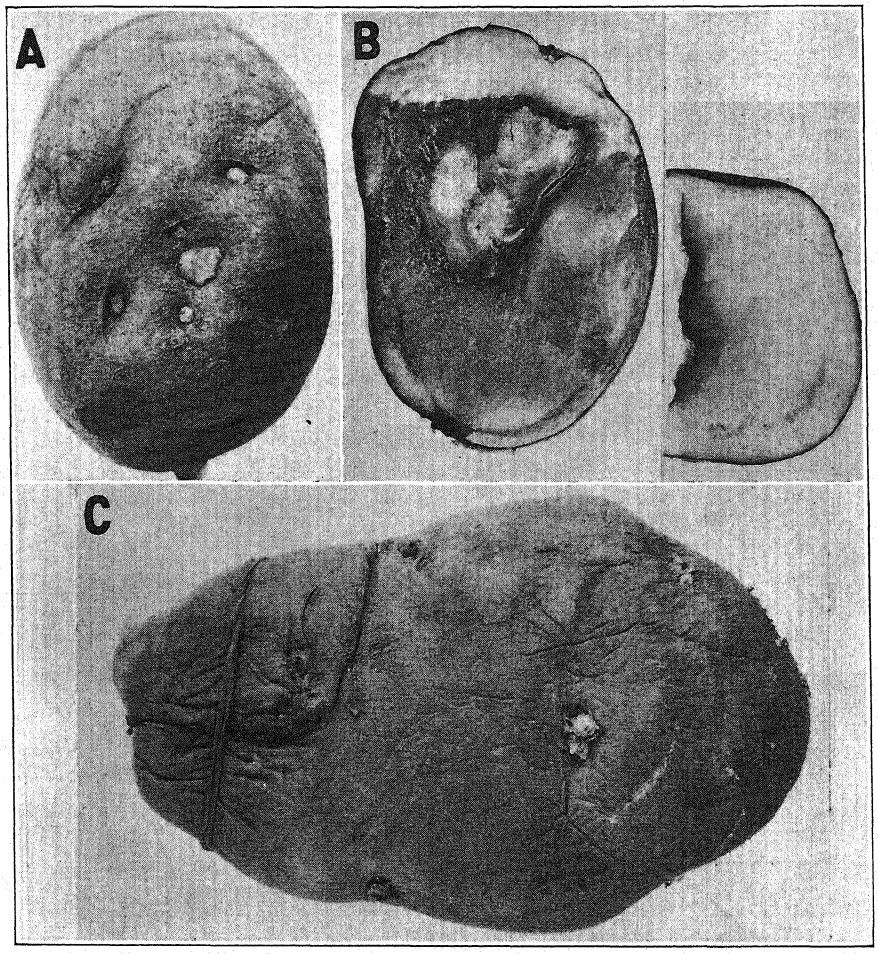


FIG. 2. Green Mountain potatoes. A. Tuber from storage. *Botrytis cinerea* had penetrated through tissues and emerged from eyes. B. Typical infection from pure culture of *Botrytis cinerea*. Left, surface, and right, section, of lesion. C. Like Fig. 1, C, except exterior view. Wrinkled where fungus had penetrated through tissue. Reisolation effected here also.

Cultures of the fungus were kindly examined by H. H. Whetzel, who wrote that it was a Botrytis of the *cinerea* type.

Pethybridge's negative results in infection experiments may have been due to his not having the advantage of certain subsequent studies of the physiology of Botrytis as a pathogen. These studies (2; 3; 4; 5; 6; 7; 8), and others on suberization in potato tubers (1, p. 996; 17, p. 28), induced the writer to use in his inoculations, vigorous young cultures in deep, fresh

TABLE 1.—*Inoculation of potato tubers with Botrytis cinerea*

Series	Month	Inoculated tubers[a]		Check tubers	Storage		Results		
		Age	No.		Temperature	Humidity	Time	Infected[b]	With deep rot
					Degrees C.			*Per cent*	*Per cent*
1	June	Old	35	35	20	Room[c]	In 14 days	60	0
2	July	Old	15	11	20	Room	In 9 days	38	0
		New	12	11	20	Room	In 9 days	25	0
3	August	Old, firm	52	17	5–20	Room or saturated	In 18 days	100	8
3a[d]					20	Room	In 8 weeks	100	17
					20	Saturated	In 8 weeks	100	50
					5	Room	In 8 weeks	100	28
					5	Saturated	In 8 weeks	100	64
4	August	Old, wrinkled	14	0	5	Room or saturated	In 18 days	100	0
							In 8 weeks	100	79
5	October	New	5	5	10	Nearly saturated	In 3 months	60	0
			25[e]	25	10	Nearly saturated	In 3 months	8	0
6	October	New	22	22	10	Nearly saturated	In one month	64	0
			12	12	10	Saturated	In one month	58	17

[a] Of Green Mountain variety except new tubers (Irish Cobbler) of series 2.
[b] No checks infected.
[c] With windows open in a generally rather humid climate.
[d] Same as 3, but at a later date.
[e] Spore inoculations, others being the usual mycelial, both kinds in this series being made in wounds only 1 cm. deep.

wounds, closed tightly after inoculation, and to keep some of the inoculated tubers in a damp chamber at about 5° C. The cultures were nonsporulating ones that had been started with spore suspensions and grown several days on hard potato agar at about 20° C. Usually every second or third tuber in order was used as a check. The checks were treated in the same way as the inoculated tubers except for the absence of any fungus on the inoculating needle. The results of the writer's inoculations are given in the following table.

It is apparent from the table, in inoculation series 3, that deep rot, meaning by this an active penetration by the rot at a depth of 1 cm. or more, was increased somewhat by a lowering of the temperature and was increased distinctly by saturating the air.

In these various inoculations there was no consistent difference between cultures originating from tubers in 1927, from leaves and stems in 1931, or from tubers in 1932. In the sixth series of inoculations, infection was produced similarly by 2 monosporic subcultures and a monohyphal subculture of a culture originating from a tuber in 1927.

For reisolation in series 1, 8 tubers were used, of which one gave a sterile plate and the others *Botrytis cinerea*. Of the latter all were pure cultures except one, which was contaminated with bacteria. These reisolates were used for the majority of the inoculations in series 3 to 6. In series 3 and 4 together, reisolation in 19 days was attempted from only 8 tubers, with positive results in pure culture in all instances. In 2 months, attempted reisolations were successful from 12 of the then tested 21 tubers. Among the remaining 9 failures were 3 that previously had given *B. cinerea,* and 5 of the failures gave as many species of Fusarium. Apparently other fungi sometimes can crowd out *B. cinerea.*

The diseased tubers found by the writer in storage in 1932 appeared to have been infected at the stem end. This, together with the previously quoted observation of Pethybridge on Botrytis tuber rot, indicates that tubers are infected chiefly or only by internal mycelium entering from infected stems through the stolons. This idea is supported further by the comparative failure of spore inoculations in the fifth inoculation series described above. In 1931, conditions favored the Botrytis blighting of potato leaves and stems in Maine so that in some fields this disease caused considerable injury and apparently caused more injury than any other. It seems probable that in the several regions characterized by the occurrence of severe *Botrytis cinerea* blight of potato foliage, namely, Maine,[3] Germany (10), Holland (16, pp. 144–146), Ireland (12; 13; 14, pp. 579–584), and England (9, pp. 42–43), close examination of tubers following such blight will dis-

[3] U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rprtr. 16: 15. 1932. (Mimeographed.)

close other instances of tuber infection. Such infection may be somewhat masked by late-blight rot and accompanying rots, due to the fact that late blight usually is epidemic in conditions favoring Botrytis blight (14, pp. 579–584).

SUMMARY

Cultures of *Botrytis cinerea* isolated from actively expanding potato-tuber lesions produced the identical disease when inoculated into potato tubers under certain conditions and produced a characteristic shallow lesion under other conditions. Similar results were obtained with cultures of this fungus that had been isolated from soft-rotted tubers several years previously, from lesions on stems and leaves, and from the tubers infected experimentally; and with monosporic and monohyphal subcultures. Mycelial cultures were much more effective than spores in producing infection. From the available data it is concluded that tuber infection probably occurs, following Botrytis blight of the foliage and stems, chiefly or solely as the result of internal mycelium entering from infected stems through the stolons.

MAINE AGRICULTURAL EXPERIMENT STATION,
ORONO, MAINE.

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PHYTOPATHOLOGICAL NOTES

Pecans Infected with Nematospora coryli Pegl.—An organism identified as *Nematospora coryli* Pegl. was isolated from brown areas on kernels of pecan nuts in Florida, in January, 1933. (Fig. 1.) The nuts from which the organism was isolated had been stored from two to three months. In pure cultures on potato-dextrose agar the organism is indistinguishable from similarly-grown cultures of *N. coryli* Pegl. previously isolated[5] from orange, grapefruit, tangerine, satsuma, and tomato in Florida. This is apparently the first time the organism has been isolated from pecans and the only report of it on any variety of nuts except hazelnuts, as reported by Peglion[4] in Italy. It has been reported from various parts of the world by pathologists[1,3,5] on more than 20 other hosts. Investigations

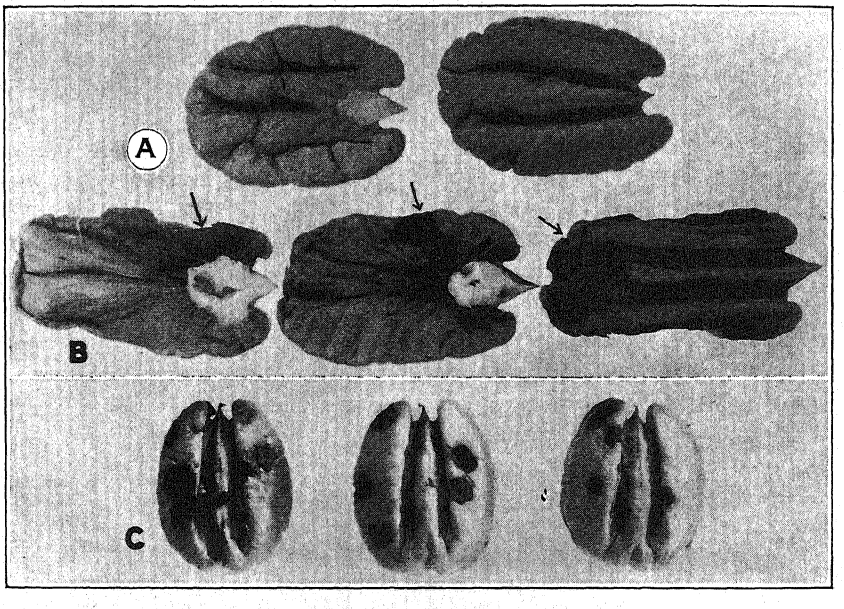


FIG. 1. A. Disease-free half kernels of pecan. B. Half kernels infected with *Nematospora coryli* Pegl. and showing shriveled and darkened tissue. C. "Kernel spot" infections produced by certain sucking insects.

[1] Ashby, S. F., and W. Nowell. The fungi of stigmatomycosis. Ann. Bot. 40: 69–83. 1926.

[3] Fawcett, H. S., and H. A. Lee. Inspissosis or dry rot of fruit. Pp. 415–420. *In* Citrus diseases and their control. 582 pp. McGraw-Hill Book Company, New York. 1926.

[4] Peglion, Vittorio. Über die *Nematospora coryli* Pegl. Centr. Bakt. Abt. 2, 7: 754–761. 1901.

[5] Weber, George F. Occurrence and pathogenicity of *Nematospora* spp. in Florida. Phytopath. 23: 384–388. 1933.

have not been made to determine whether this organism is involved in the shedding of nuts, which is often considerable, during the growing season.

The infection cannot be detected until the shells are removed, when it is found on the convex outer portion of the kernels, usually originating on the tops of the convolutions or on the edges of the half kernels. This is also true to a large extent of the discolored areas known as "kernel spot," which has been shown[2] to be caused by certain sucking insects. The brown color of the diseased area is very similar to the dark brown to black color of kernel spot, and in most instances the two are associated. The areas are slightly sunken and shrunken, showing a visible margin. The tissue below the diseased area is punky and is slightly brownish-stained.

The mode of infection is of primary interest and until experimental data are available, it will be only hypothetical. Insects of the species causing kernel spot have been found feeding on numerous hosts, and *Nematospora coryli* has been isolated from necrotic areas on a score of them. It is very probable that the organism is carried by these sucking insects, resulting in the infection of the pecan kernels.—GEORGE F. WEBER, University of Florida, Gainesville, Florida.

On the Pathogenicity of Wojnowicia graminis.—In Australia, Pittman[1] and others state that *Wojnowicia graminis* (McAlp.) Sacc. causes a root rot of wheat plants. It is also reported as a wheat parasite in Europe by Guyot,[2] Foëx and Rosella,[3] Van de Laar,[4] and others.

While *Wojnowicia graminis* apparently is considered as a parasite on wheat plants in Australia and Europe, it is thought to be a very weak pathogen, at best, in North America. Apart from the statements of McKinney[5] in the United States and Fraser[6] in Canada, that *W. graminis*

[2] Demaree, J. B. Kernel-spot of the pecan and its cause. U. S. Dept. Agr. Bul. 1102. 1922.

[1] Pittman, H. A. Some parasitic and non-parasitic causes of partial or complete emptiness in wheat heads. Dept. Agr. W. Aust. Bul. 301. 1930. (Reprint of: Some parasitic and nonparasitic causes of "empty" or "tipped" heads in wheat. Jour. Dept. Agr. W. Aust. II, 7: 153–164. 1930.)

[2] Guyot, L. De l'existence de formes pycnidiennes chez *Ophiobolus graminis,* Sacc. et *Ophiobolus herpotrichus* (Fr.) Sacc. Rev. Path. Vég. et Ent. Agr. 12: 74–81. 1925.

[3] Foëx, E., and E. Rosella. Sur les diverses formes du piétin. Rev. Path. Vég. et Ent. Agr. 17: 41–51. 1930.

[4] Van de Laar, J. H. J. Onderzoekingen over *Ophiobolus graminis* Sacc. en *Ophiobolus herpotrichus* (Fr.) Sacc. en over de door deze fungi veroorzaakte ziekten van *Triticum vulgare* Vill. en andere Gramineae. Thesis, Wageningen Agricultural College. 146 pp. 1931.

[5] McKinney, H. H. Foot-rot diseases of wheat in America. U. S. Dept. Agr. Bul. 1347. 1925.

[6] Fraser, W. P. *Wojnowicia graminis.* Rpt. Dominion Botanist (Canada) 1924: 71. 1925.

is only very slightly pathogenic to wheat, there appear to be no very definite experimental data on the subject, except those in Europe reported by Guyot,[2] Foëx and Rosella,[3] and Van de Laar.[4] Therefore, since *W. graminis* is very common in Alberta on the basal parts of both winter and spring wheat, where it frequently is associated with *Ophiobolus graminis* Sacc., it seemed desirable to secure experimental data in this connection.

Single-spore isolations of *Wojnowicia graminis* from pycnidia obtained from wheat plants, growing at 6 widely separated points in Alberta, and 28 isolations of the fungus, obtained from the crowns of wheat plants, taken at random in a field, were tested for virulence on Kharkov, a winter wheat, and Marquis, a spring wheat. The tests were made in the greenhouse in sterilized soil, at a temperature range of 16° to 18° C. Ten grams of inoculum grown on sterilized oat-hull media was placed at seed level in each pot. Thirty wheat plants of each variety were used to test each culture. The plants were examined for disease when 40 days old.

The data secured indicated that none of the 34 isolations of *Wojnowicia graminis* mentioned were significantly pathogenic under the conditions of the experiment. Plants in the sterilized infested soil were as healthy as those grown in sterilized soil. Whether this fungus is pathogenic during the post-seedling stage, under field conditions, must remain an open question until settled experimentally. However, under greenhouse conditions, there was still no apparent evidence of pathogenicity when the plants of the 2 varieties of wheat grew to maturity.

In addition to the pathogenicity tests, measurements were obtained of 100 spores from a pycnidium of *Wojnowicia graminis* on a winter-wheat plant at Cowley, Alberta. The average size was 36.0×3.9 μ, the range being 26.3 to 43.8 μ and 2.5 to 5.6 μ, respectively. The average number of septations was 6.8, with a range of 3 to 8. The data indicate that the spores of *W. graminis* mentioned were slightly larger and wider than those mentioned by Van de Laar, viz., 29.58×3.47 μ, with 5 to 7 septations.—W. C. BROADFOOT, Dominion Laboratory of Plant Pathology at University of Alberta, Edmonton, Canada.

Another Powdery Mildew on Crape Myrtle.—Powdery mildew of crape myrtle has been known in the Gulf States since 1925 when Taubenhaus found it in Texas. In a recent paper[1] the perfect stage of this common crape-myrtle mildew was described as *Erysiphe lagerstroemiae,* n. sp., and the statement was made that no perithecia of any other powdery mildew had been found on this host in the United States. Since that paper was submitted for publication, specimens have been sent to me for identification

[1] West, Erdman. Powdery mildew of crape myrtle caused by *Erysiphe lagerstroemiae,* n. sp. Phytopath. 23: 814–819. 1933.

by Dr. G. W. Carver, of Tuskegee, Alabama, which prove to be *Phyllactinia corylea* (Pers.) Karst. The mycelial growth of these specimens is sparse and patchy, but the few perithecia present are quite typical. However, the general aspect of the mildew caused by Phyllactinia is quite different from that of the common crape-myrtle mildew. In the former only a thin, patchy growth of mycelium and conidia occurs; in the latter there is a heavy and general growth of mycelium and conidia that covers the leaves and stems with a thick, mealy coating. In the numerous collections of powdery mildew on crape myrtle examined in Florida, no perithecia of Phyllactinia have ever been found. Since Phyllactinia is rather common in this State on several other hosts, it seems unlikely that its occurrence on crape myrtle would have passed unnoticed, unless it were rare. Further observations are desirable, especially on the common crape-myrtle mildew, which was reported for years under the name of *Uncinula australiana* McAlp., either with or without an interrogation point.—ERDMAN WEST, Florida Agricultural Experiment Station, Gainesville, Florida.

Rawlins, Thomas Ellsworth. *Phytopathological and Botanical Research Methods.* ix + 156 pp., 3 figs. John Wiley & Sons, Inc., New York. 1933. Price $2.50.

Methods of research and technique of plant pathology, botany, and other biological sciences are published in a large number of books, journals of all kinds, and often in obscure periodicals not generally read. Investigators, therefore, will welcome any attempt to bring together in a single volume in an orderly arrangement the methods that have been found useful in research work. The volume bearing the above title is an attempt in this direction. The author has not only drawn generously from the publications of research workers, but he has made liberal use of methods developed by himself as a teacher and investigator. The book is divided into three parts as follows: Part I. Choosing and Planning a Research Project; Part II. Experimental Methods; Part III. Interpretation of Experimental Results.

Parts I and III are both short and probably contribute least to the value of the book. It is not easy to tell an investigator how to choose a project for work, if indeed he has any choice in the matter, or how to interpret the results after they are obtained. Part II, on the other hand, includes a mass of data and serves as a source of information on methods and technique. The book spares the investigator either the necessity of memorizing the various methods to be applied in any particular line of work or that of searching for them in the published works of others.

The author has probably made his greatest contribution in the chapter entitled "Microscopic Methods," and especially that part under microchemistry. Here he has collected in a concise form methods that may be employed by the investigator as type material in his comparative work. His statements and discussions are supported by citations of the work of other investigators in a similar field. As complete as the book is, some subjects regarded by the reviewer as important, unfortunately, have been omitted or only inadequately treated. For example, under culture media methods especially applicable for use in growing cultures to be used in soil inoculations are entirely disregarded. Again, under sterilization methods nothing is said about soil sterilization that is likely to be more difficult than the mastery of the autoclave to which he gives a paragraph. With the increasing interest and importance of diseases caused by soil-inhabiting organisms, a knowledge of soil sterilization and soil inoculation and management is especially important, yet this phase of the subject is sparingly discussed. The very important subject of inoculating plants is given less than two pages.

This phase of phytopathology is highly important and more space could profitably have been given to a fuller discussion of methods and technique.

The failure to obtain infection is often due not to a lack of parasitism of a particular organism but to a lack of the proper relation of the host with respect to temperature and humidity. Temperature and water content of the soil also are governing factors in the infection by soil organisms. The importance of air temperature and moisture and temperature and water content of the soil in relation to infection and methods for their control might very profitably have been treated at some length.

The book closes with 960 citations and an excellent index. There is no question of the amount of work involved in working through such a large number of articles and extracting therefrom methods that contribute to phytopathological science. The citations are well chosen and themselves classified under—1, Histology and Cytology; 2, Microchemistry; 3, Cultural Methods; 4, Viruses; 5, Miscellaneous Methods; 6, Interpretation of Experimental Results.

On the whole the book is excellent and deserves, for ready reference, a place on the shelf of all phytopathologists and others engaged in botanical research.—L. L. HARTER, Washington, D. C.

Raber, Oran. *Principles of Plant Physiology.* vii + 432 pp., 29 figs. Edition II Revised, Macmillan Co. New York. 1933. Price $3.50.

This is a revised and enlarged edition of the excellent elementary text, which was first published in 1928. The number of chapters has been increased from 31 to 33 and the number of pages from 377 to 427. The chapter headings remain very much the same as they were in the first edition, except the chapters on Growth and Reproduction. The former has been divided into two chapters, "General Characteristics of Growth" and "Specific Growth Factors" and the latter into two chapters "Reproduction" and "Reproductiveness and Seed Germination." New sections have been added on hydrogen-ion concentration and photoperiodism, while rather extensive additions have been made to other topics in discussing them more intensively or in bringing them up to date.

It is always a question to the teacher of any subject how much of related matters should be included in the presentation of the material in his special field. It is thus a moot question how much of subjects that are vehicles of expressing physiological facts should be discussed in a text on plant physiology. It is doubtful if colloids and hydrogen-ion concentration can be so adequately expressed as to be of much benefit to a student in a page or two of an elementary text on plant physiology. A student who elects or is required to take elementary physiology should have been exposed sufficiently

to these topics in a general way in elementary chemistry so that little or no mention of them should be necessary.

To the reviewer, it seems that, in the discussion of the cell in Chapter II, too much emphasis and space are devoted to the subject of colloids and too little to the morphological entities of the cell. The relationships of colloidal behavior to the activities of the cell have been much over-emphasized by plant physiologists, for, in the last analysis, our knowledge of these relationships is very meager indeed.

The references to literature made in the text simply by citing the author's name and the date, without their being listed elsewhere in the book, are very confusing to those who desire to inform themselves further on the topic under consideration. Few indeed, if any, are the teachers who can recall from their knowledge of the literature the significance or importance of these citations by such abridged references. A footnote simply stating the volume and page of the reference would be an invaluable aid to the teacher as well as to the student.

The adverse criticisms that have been offered should, however, be given only minor consideration in view of the excellent presentation in an elementary way of the various topics of plant physiology. The writer knows from experience that it is much easier for a reviewer to criticize a text adversely than it is to write a text that is above criticism.

The author shows the aptitude of the teacher by missing no opportunity to cite the relationships of his subject to human affairs. This is one of the keys that always unlocks the door to the interest of the student. The list of thought-provocative questions at the end of each chapter is well chosen and will further incur the interest of the student in the subject.

The text throughout is written in a clear and concise manner and in a simple style that is easy for a student to follow. The context is well-balanced and no undue emphasis is placed on any topic, while the whole field of plant physiology is brought before the reader.

The advanced student in plant physiology would do well to read the text, for, by so doing, he would obtain a well-presented summary of that field.

The book in its content and in the manner of its presentation is a valuable contribution to the literature of elementary plant physiology.—EDWIN C. MILLER, Kansas State College, Manhattan, Kansas.